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# DETERMINATION OF CAROTENE AND VITAMIN A IN MILK

F. R. OLSON, D. M. HEGSTED, AND W. H. PETERSON
*Department of Biochemistry, College of Agriculture, Madison, Wisconsin*

Spectrophotometric methods have been used extensively for the determination of carotene and vitamin A. As far as we are aware, however, the estimation of vitamin A in milk by measurement of the absorption at 328 mμ has only been accomplished by the use of butterfat as a starting material (1, 2). This procedure is inconvenient and laborious since large samples of milk are required and the cream must be separated and churned. Wilkie (3) has outlined a method for the estimation of carotene and vitamin A in evaporated milk based upon the Roese-Gottlieb (4) method for the determination of fat in milk. In our experience the proportion of solvents specified by Wilkie did not extract the carotenoids from whole milk, but by modifying the procedure somewhat satisfactory results have been obtained.

While the data were being prepared for publication a similar extraction procedure by Willstaedt and With (5) has appeared. These authors did not use spectrophotometric methods, however, but determined both carotene and vitamin A with the step-photometer. The reading for vitamin A was based on the color developed with antimony trichloride.

## PROCEDURE

In a separatory funnel 100 cc. of milk are placed, the following reagents are added successively and the solution is shaken vigorously after each addition: 15 cc. concentrated $NH_4OH$, 100 cc. ethyl alcohol (aldehyde-free), 75 cc. ethyl ether (peroxide-free), and 25 cc. of Skelly solve B (petroleum ether b.p. 60–70°). After 20 minutes the lower layer is drawn off and is again extracted with a mixture of 25 cc. ethyl ether and 10 cc. Skelly solve B. The combined extracts are allowed to stand in the separatory funnel for 30 minutes. A considerable amount of suspended matter settles out and should be drawn off; otherwise the results will usually be too high.

The extract is transferred to a distillation flask equipped with a small separatory funnel and the flask is placed in a water bath. The solvents are

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removed under reduced pressure with the temperature of the bath no higher than 70° C. Care should be taken that the level of the liquid in the flask is not below that of the water in the surrounding bath and that the heating is not continued after the solvents have been removed. Immediately after the solvents have come off, 10 cc. of ethyl alcohol are run in from the separatory funnel before the vacuum is released.

The liquid is transferred with an additional 35 cc. ethyl alcohol to a flask connected to a reflux condenser with ground glass joints. Two and one-half grams of potassium hydroxide are added and the fat is saponified by refluxing in an atmosphere of nitrogen for 20 minutes. After saponification 100 cc. of water are added and the mixture is cooled to 4° C. The unsaponifiable matter is extracted with three portions of ether, 75, 25, and 25 cc. The ether extracts are combined in a separatory funnel and washed with four portions of cold water, 100, 100, 50, and 50 cc. The first two washings should be done by gently rotating the funnel; the last two by vigorous shaking. The water in the ether is then frozen out by placing the flask on crushed dry ice. The solution is filtered through cotton and the flask and cotton are washed with cold ether. The filtrate and washings are placed in a distilling flask and the ether is removed under reduced pressure.

Special care should also be taken in this distillation. The temperature of the water bath should not exceed 70° C. and the flask should be agitated continuously as the last traces of solvent evaporate. Just as the last of the ether boils off about 5 cc. of methyl alcohol (aldehyde-free) is run into the flask from the separatory funnel before the vacuum is released. If the residue is allowed to dry out before adding the methyl alcohol the resulting solution may be turbid and difficult to clarify. The solution is made up to 10 cc. with methyl alcohol and any precipitate present is removed by centrifuging. The carotene content of this solution is determined with the visual spectrophotometer and the vitamin A as described by Baumann and Steenbock (1).

## RESULTS

The carotene and vitamin A contents of several milks were determined by the above procedure and compared with results obtained by analysis of the butterfat from the same milks. The butterfats were analyzed by the method of Semb, Baumann, and Steenbock (6). Table 1 summarizes the results obtained. For samples 1–5 the amount of filtered butterfat weighed out corresponded exactly to 100 cc. of milk. In samples 6D and 6E an additional amount of fat was added to the milk. The source of the fat varied as noted in the table. It was dissolved in the 100 cc. of ethyl alcohol used in the extraction. In one case, 6D, the added butterfat was obtained from the same batch of milk as was analyzed, so the carotene and vitamin A calculated on a butterfat basis should not have changed. The calculated carotene content was 2.8, found was 3.0; calculated vitamin A was 8.9, found was 8.8.

TABLE 1

*Comparison of milk and butterfat as starting material for the determination of carotene and vitamin A*

| Number of sample | Description of sample | Material analyzed | Butterfat in sample | Carotene (based on butterfat) | Vitamin A (based on butterfat) |
|---|---|---|---|---|---|
| | | | gm. | γ/gm. | γ/gm. |
| 1A | Mixed market | Milk | 4.1 | 4.4 | 8.5 |
| 1B | " " | Butterfat | 4.1 | 4.7 | 8.5 |
| 2A | Mixed market | Milk | 3.7 | 3.9 | 9.6 |
| 2B | " " | Butterfat | 3.7 | 3.7 | 9.6 |
| 3A | Guernsey | Milk | 4.3 | 5.3 | 7.3 |
| 3B | " | Butterfat | 4.3 | 5.2 | 7.3 |
| 4A | Certified mixed | Milk | 4.3 | 6.9 | 7.8 |
| 4B | " " | Butterfat | 4.3 | 6.3 | 7.9 |
| 5A | Mixed market | Milk | 3.7 | 3.8 | 8.7 |
| 5B | " " | Butterfat | 3.7 | 3.3 | 9.5 |
| 6A | Holstein | Butterfat | 15.0 | 2.9 | 8.5 |
| 6B | " | Milk | 3.4 | 2.5 | 9.3 |
| 6C | " | " | 3.4 | 2.8 | 9.3 |
| 6D | " | " * | 6.4 | 3.0 | 8.8 |
| 6E | " | " † | 6.4 | 5.4 | 9.3 |
| 7A | Mixed market | Milk | 4.5 | 8.3 | 9.6 |
| 7B | " " | " | 4.5 | 8.1 | 9.6 |
| 7C | " " | Butterfat | 15.0 | 8.2 | 9.5 |
| 7D | " " | " | 4.5 | 8.6 | 9.6 |
| 7E | " " | " | 3.0 | 8.6 | 9.6 |

* Plus 3 gm. butterfat prepared from milk No. 6B.
† Plus 3 gm. butterfat prepared from milk No. 7A.

The butterfat added to the milk in 6E came from a different milk and here again the recovery was good; carotene, calculated 5.4, found 5.4; vitamin A calculated 9.4, found 9.3.

The amount of butterfat used as starting material did not affect the final results. In samples 7C, D, and E, the amount of fat used varied from 3 to 15 grams, but the carotene figure varied only 0.4 of a microgram and the vitamin A only 0.1 microgram per gram of butterfat. These checks are as good as duplicate determinations.

Often it was impossible to assay the milk samples as soon as they were received. In order to see if storage might affect the determination runs

TABLE 2

*Effect of storage and freezing of milk on carotene and vitamin A content*

| No. of analysis | Treatment of milk | Carotene (based on butterfat) | Vitamin A (based on butterfat) |
|---|---|---|---|
| | | γ/gm. | γ/gm. |
| 1 | None | 6.0 | 7.5 |
| 2 | Stored two weeks at 12° C. | 6.2 | 6.7 |
| 3 | Three weeks at 12° C. then frozen for 10 days | 6.2 | 7.4 |
| 4 | Same as No. 3 then stored 14 days again at 12° C. | 6.0 | 7.2 |

were made on a certified sample immediately after it was received and later after storage under various conditions and for various periods of time. It was allowed to stand in an ice box for two weeks before a second analysis was made. It was stored in an ice box for another week and was then kept frozen for 10 days before the third determination was made. It was again stored in an ice box for another two weeks before the fourth analysis was made. The results of these analyses are given in Table 2 and show that storage and freezing apparently did not affect either the carotene or the vitamin A content of the milk.

## SUMMARY

A method has been developed for determination of carotene and vitamin A that can be applied directly to milk. The results check well with those obtained when butterfat is used as the starting material.

Milk can be stored or frozen for considerable periods without affecting the carotene or vitamin A content.

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# PRODUCTION OF DIACETYL FROM CITRIC ACID IN BUTTER CULTURES[1]

E. A. PRILL[2] AND B. W. HAMMER

In the normal ripening of a butter culture the important fermentation products come from two main sources, lactose and citric acid. The former yields lactic acid while the latter yields a series of neutral compounds (diacetyl, acetylmethylcarbinol and 2,3-butylene glycol), together with acetic acid and carbon dioxide. Lactic acid ordinarily is produced in ample amounts unless there is some irregularity in the culture, such as the presence of an active bacteriophage, but the products formed from citric acid are often in such low concentrations that the culture lacks flavor and aroma, and does not have the desired effect when used in butter manufacture.

Supplementing the small percentage of citric acid normally in milk by addition of this acid to milk used for butter culture results in a conspicuous increase in the acetylmethylcarbinol plus diacetyl content (4, 5) and, in accordance with this, the flavor of the butter made with such culture is improved (2, 8).

Although diacetyl is a very important aroma substance of butter cultures and butter, in most of the work reported it was not determined separately because of inadequacy of the methods available for determining the small amounts present. The colorimetric method recently developed by Prill and Hammer (7) readily permits the determination of minute quantities of diacetyl. It was used in studies on the fermentation of citric acid in butter cultures with special reference to production of diacetyl. The results are reported herein.

## ANALYTICAL METHODS

The diacetyl was determined colorimetrically (7) by converting it into dimethylglyoxime and then into the intensely colored ammono-ferrous dimethylglyoximate. In determining acetylmethylcarbinol plus diacetyl, the sample with ferric chloride added was distilled as in the usual procedure (5); a small aliquot of the distillate was taken, additional hydroxylamine acetate added to maintain the proper concentration of this reactant when diluted to 6 ml., and the procedure then continued in exactly the manner used for the diacetyl. Actually, the values recorded for acetylmethylcarbinol plus diacetyl are the amounts of diacetyl obtained after oxidation with ferric chloride. Various workers have noted that this oxidation is

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[2] The studies reported were largely financed through a grant from Chas. Pfizer and Co., Inc., which provided particularly for the services of Dr. Prill.

not absolutely quantitative and for this reason a correction for the slight difference in molecular weights is not warranted.

All the results on diacetyl and acetylmethylcarbinol plus diacetyl are expressed as parts per million (p.p.m.).[3]

## GENERAL METHODS OF PREPARING BUTTER CULTURES

The butter cultures studied were prepared from well pasteurized skim or whole milk by inoculating with one of the butter cultures carried in the laboratory. In some cases citric acid (the monohydrate was regularly used) was added to the milk as an aqueous solution with vigorous agitation (to avoid excessive coagulation). Depending on the type of experiment, the milk was either divided into a number of portions and the portions ripened at 70° F. for about 16 hours, or an entire volume was ripened and any desired division then made.

Further treatments of the butter cultures are given under the different series of trials.

## RESULTS

Data on the effects of various treatments on the diacetyl and the acetylmethylcarbinol plus diacetyl contents of butter cultures are presented in four series of trials. In each series the trials were repeated until the general changes occurring appeared to be well established; for each series representative data are reported rather than all the trials.

In discussing the results, changes in the acetylmethylcarbinol plus diacetyl contents are referred to simply as increases or decreases in acetylmethylcarbinol (*the* carbinol); this is sufficiently exact, especially when the diacetyl contents are known, because acetylmethylcarbinol makes up such a large percentage of the total acetylmethylcarbinol plus diacetyl.

### *Series 1. Effects of Varying Amounts of Citric Acid Used in the Preparation of Butter Culture on Ripened Cultures Held Cold*

The cultures were prepared in flasks with addition of different amounts of citric acid (0.00 to 0.30 per cent) to the milk. After ripening, the cultures were held in the cooler (36° to 45° F.). At intervals (usually successive days) each of the flasks was shaken and a portion of the contents removed for analysis. Data for two trials are given in Figures 1 and 2.

When analysed on the first day (about 10 hours after the ripened cultures had been placed in the cooler), the cultures with added citric acid regularly had higher diacetyl contents than those without added citric acid.

[3] In various publications from this laboratory the diacetyl and the acetylmethylcarbinol plus diacetyl values are expressed as milligrams of nickel dimethylglyoximate obtained from a 200 or a 400 g. sample. For comparison, such results can be converted to p.p.m. of the actual fermentation products by multiplying the value for a 200 g. sample by 3 and the value for a 400 g. sample by 1.5.

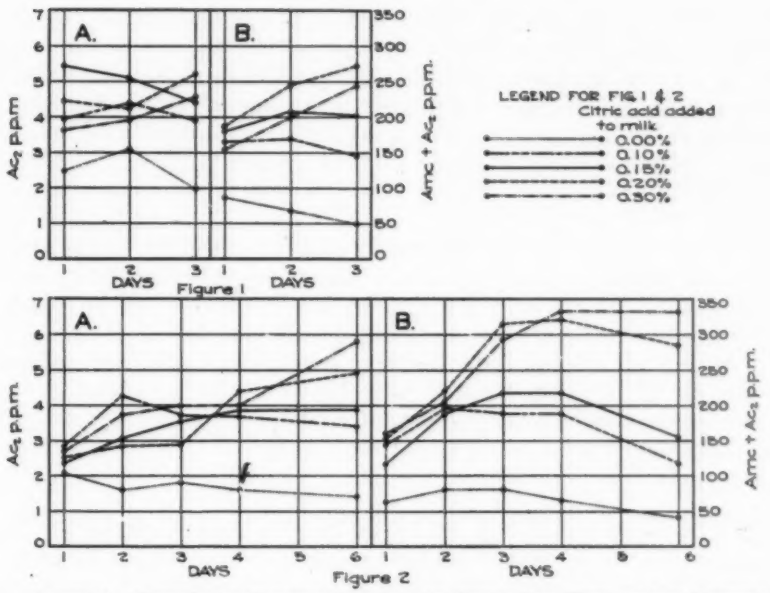



Figure 1




Figure 2

SERIES 1. EFFECTS OF VARYING AMOUNTS OF CITRIC ACID USED IN THE PREPARATION OF BUTTER CULTURES ON RIPENED CULTURES HELD COLD.—A. Values for diacetyl ($Ac_2$). B. Values for acetylmethylcarbinol plus diacetyl ($Amc. + Ac_2$).

The increases in diacetyl showed no relationship to the amounts of citric acid employed; in Figure 1 the diacetyl content was highest with 0.15 per cent added citric acid and next to the lowest with 0.30 per cent, while in Figure 2 there were only slight differences in the increases resulting from the various amounts of citric acid. During succeeding days the diacetyl contents of the cultures prepared with the larger amounts of citric acid tended to increase, whereas those prepared with the smaller amounts or none tended to increase at first but often decreased later. The maximum increases in diacetyl of the cultures prepared with the highest concentration of citric acid seemed to be delayed, probably because of slow development of the organisms in a medium which was relatively high in acid when inoculated. At the close of the periods studied, the diacetyl contents were in the same orders as the concentrations of citric acid employed, except with 0.30 per cent in Figure 1.

On the first day the carbinol contents were regularly higher with added citric acid, but the increases again showed no relationship to the amounts of citric acid used. The retarding influence of 0.30 per cent acid was evident, especially in Figure 1. With longer holding the acetylmethylcarbinol contents increased significantly with time, reached a maximum and

then decreased, as is well illustrated in Figure 2; presumably the decrease occurred when all the citric acid has been utilized and the carbinol was being converted into such substances as 2,3-butylene glycol and possibly acetic acid. It appears that the larger the amount of citric acid present, the higher the maximum production of the carbinol and the longer the period before the decrease began. If the trial shown in Figure 1 had been extended, the further changes probably would have been somewhat similar to those shown in Figure 2.

The differences in the diacetyl and the carbinol contents in the two trials may have been due to differences in the characteristics of the cultures used. The possible irregularity of some of the data in either of the trials may have been caused by non-uniform shaking of the samples.

In previous studies ripened cultures were usually held for extended periods at 70° F. The results given in Figures 1 and 2, and also in the later figures, show that very substantial increases in both the diacetyl and the acetylmethylcarbinol contents can occur at considerably lower temperatures also.

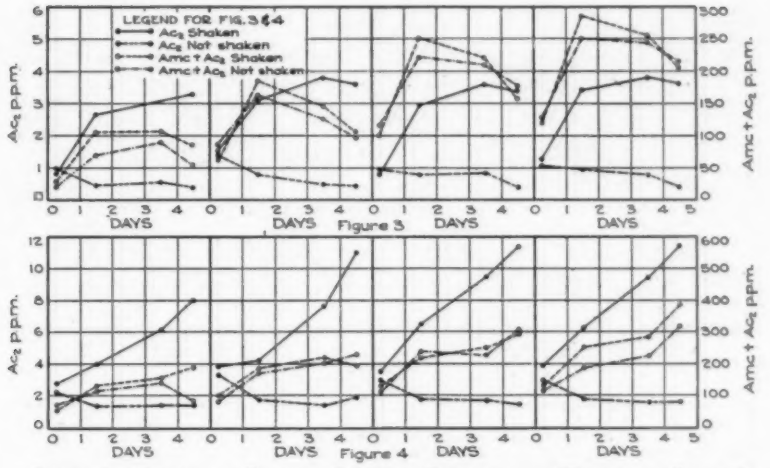
*Series 2. Effects of Shaking Cultures Prepared and Held as in Series 1*

Brewer *et al.* (1) found very marked increases in the diacetyl contents by growing butter culture under aeration and pressure, and Virtanen (9) recommended the ripening of butter cultures in shallow containers to allow greater access of air. In analyses (not reported) incidental to the present work it was frequently observed that merely shaking an active culture resulted in an appreciable increase in diacetyl within a relatively short time.

To study the effect of shaking on cultures, trials similar to those in Series 1 were made, but with the difference that in place of only one flask for each concentration of citric acid there were four bottles which were undisturbed until analyzed, as well as one flask from which successive portions were removed for analysis and the remainder then vigorously shaken. The first analyses were made when the cultures had been in the cooler about 6 hours. The results are given in Figures 3 and 4.

The most important points are that the diacetyl contents of the shaken samples increased progressively even without added citric acid, whereas those of the corresponding unshaken samples decreased and then remained at a low level, and that there were relatively small differences in the acetylmethylcarbinol contents of the corresponding shaken and unshaken samples although in most cases the latter were the lower. The initial contents of diacetyl and of the carbinol and the changes in the carbinol contents are in general accord with those of Series 1.

The comparatively high diacetyl contents shown in Figure 4 were obtained with butter culture 146, which has repeatedly produced unusually high diacetyl contents under certain conditions; in practice this culture is considered to be coarse, rather than delicate, in flavor.




SERIES 2. EFFECTS OF SHAKING CULTURES PREPARED AND HELD AS IN SERIES 1.—The four successive parts of each figure show the values for the diacetyl ($Ac_2$) and the acetylmethylcarbinol plus diacetyl (Amc. + $Ac_2$) when 0.00, 0.10, 0.20, and 0.25 per cent citric acid were used in the preparation of the butter culture.

### *Series 3. Effect of Periodic Shaking, with or without Simultaneous Addition of Citric Acid, on Ripened Cultures Held Cold*

For each trial two large flasks of milk were inoculated, one with no citric acid added to the milk and the other with 0.15 per cent added. Each flask of milk was divided among as many bottles as were required for the analyses to be made. The bottles were incubated at 70° F. and then placed in the cooler. Some of the bottles were given no further treatment, others were shaken, and still others were shaken with addition of 0.08 per cent citric acid; an attempt was made to keep the shaking uniform. The time and nature of these subsequent treatments and the results obtained are indicated in Figures 5, 6, 7, and 8. The analyses on the first day were made from 6 to 10 hours after placing the samples in the cooler. In these figures the analytical results for each sample are shown in a circle. The circles are indicated in perspective as being at different levels to suggest the relative values of the diacetyl contents most commonly obtained under the various conditions indicated. Naturally, a few of the diacetyl values in the individual trials show departures from the general tendencies, and also the changes in the acetylmethylcarbinol contents frequently do not parallel the changes in the diacetyl contents.

The diacetyl contents of the cultures held without treatment often increased somewhat from the first to the second day and in some cases to the third day. This may have been influenced by a small amount of oxygen

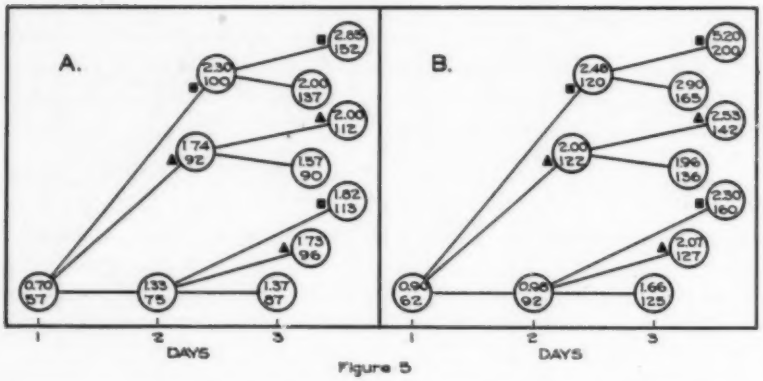



Figure 5

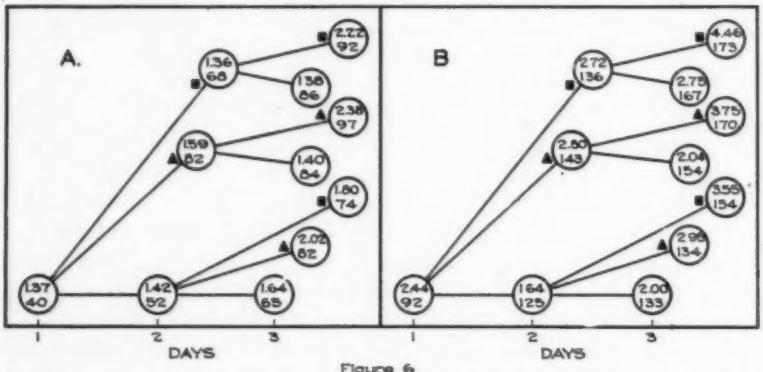



Figure 6

▲ Shaken one day before analysis ■ 0.08% Citric acid added with shaking one day before analysis

SERIES 3. EFFECTS OF PERIODIC SHAKING, WITH OR WITHOUT SIMULTANEOUS ADDITION OF CITRIC ACID, ON RIPENED CULTURES HELD COLD.—A. No citric acid added to original milk. B. 0.15 per cent citric acid added. Top figure in each circle = p.p.m. diacetyl and bottom figure = p.p.m. acetylmethylcarbinol plus diacetyl.

still present and possibly by other factors. The cultures shaken on the first day showed markedly increased diacetyl contents on the second day and part of such increases were usually retained on the third day; when shaken on both the first and second days there were still higher diacetyl contents on the third day. The cultures shaken with the addition of citric acid on the first day gave greatly increased diacetyl contents on the second day which were commonly retained on the third day; by using this treatment on both the first and second days the highest diacetyl contents were usually attained on the third day. In most cases the cultures prepared with citric acid added to the milk originally had higher diacetyl contents than the

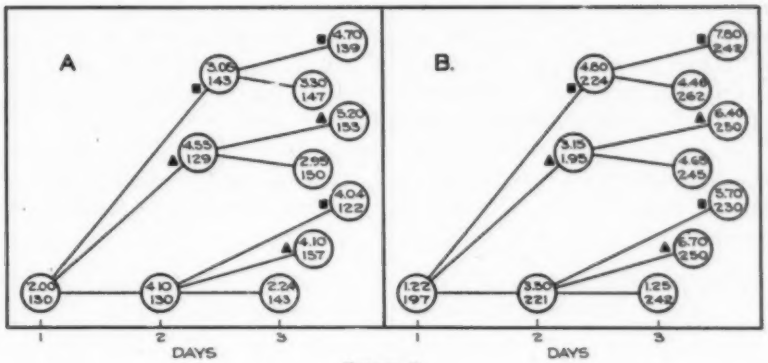



Figure 7

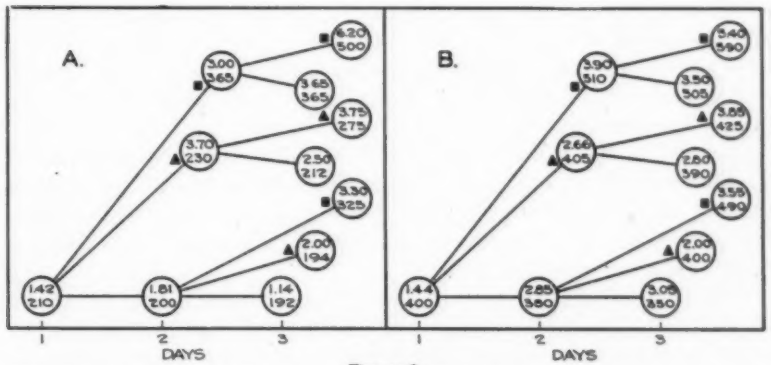



Figure 8

▲ Shaken one day before analysis ■ 0.08% Citric acid added with shaking one day before analysis

**Series 3, *continued*. Effects of Periodic Shaking, with or without Simultaneous Addition of Citric Acid, on Ripened Cultures Held Cold.**—A. No citric acid added to original milk. B. 0.15 per cent citric acid added. Top figure in each circle = p.p.m. diacetyl and bottom figure = p.p.m. acetylmethylcarbinol plus diacetyl.

corresponding cultures without such addition. Some of the samples held without treatment subsequent to ripening were among the exceptions; in these cases the available oxygen rather than the citric acid may have been the limiting factor.

The acetylmethylcarbinol contents increased during holding without any treatment, but shaking or shaking with addition of citric acid usually gave additional increases. The increases in the carbinol contents that resulted from shaking may have been due to prevention of reduction to 2,3-butylene glycol (3), although there are other possibilities.

For the data shown in Figure 8 the ripened cultures were held at a slightly higher temperature (42° to 50° F.) than usual. The high acetyl-

methylcarbinol contents on the first day suggest that there already had been extensive utilization of the citric acid. The subsequent addition of citric acid caused very substantial increases in both diacetyl and the carbinol but especially in the carbinol; presumably, this was due to the higher temperature and the greater activity of the organisms used.

Relatively high diacetyl contents are shown in Figure 7. These were again obtained with butter culture 146. However, the acetylmethylcarbinol contents were not greatly influenced by the treatments subsequent to ripening.

### *Series 4. Effects of Adding Citric or Sulfuric Acid to Ripened Butter Cultures Held at 70° F. or Packed in Ice*

For each trial butter culture was prepared with and without addition of citric acid to the original milk. Each lot of ripened culture was then divided among a number of bottles and various amounts of citric or sulfuric acid added to the different bottles with uniform shaking. The pH values were determined with a quinhydrone electrode. Some of the bottles in each trial were returned to 70° F. and some were packed in ice. The data are given in Table 1.

Two trials, 1 and 2, show the effects of 0.15 per cent citric acid added to the milk used for the cultures. The various treatments of the ripened cultures commonly gave higher diacetyl and acetylmethylcarbinol contents when an original addition of citric acid had been made. These results substantiate those already presented.

With the addition of citric acid to the ripened cultures held at 70° F., both the diacetyl and the carbinol contents were progressively higher the greater the percentage of citric acid added; when the cultures were held in ice the diacetyl contents usually were even higher than those of the corresponding samples held at 70° F., but there commonly was relatively little production of the carbinol.

The addition of sulfuric acid to the ripened culture held at 70° F. resulted in increases in the diacetyl and in the carbinol, but these increases were considerably less than those in cultures having similar initial pH values as the result of adding citric acid. However, when the samples with sulfuric acid were held in ice the diacetyl contents were often higher than the corresponding values obtained with citric acid.

The results with sulfuric acid emphasize the importance of pH from the standpoint of production of diacetyl and also of the carbinol, since sulfuric acid cannot be attacked by the organisms. Michaelian, Hoecker and Hammer (6) found that with milk cultures of citric acid fermenting streptococci acidified with certain acids there were definite optimum pH ranges (3.9 to 3.7 with citric, 4.4 to 4.1 with lactic and 3.6 to 3.2 with sulfuric acid) for the maximum production of the carbinol plus diacetyl

TABLE 1

*Effect of adding citric or sulfuric acid to ripened butter cultures held at 70° F. or packed in ice on diacetyl ($Ac_2$) and acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) contents*

| Trial no. | | 1 | | | | | | 2 | | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | a | | | b | | | a | | b | | | | | | | | | | |
| Butter culture used, no. | | 15/1 | | | | | | 232 | | | | 232 | | | 232 | | | 15/1 | | |
| Citric acid added before ripening culture | | none | | | 0.15 per cent | | | none | | 0.15 per cent | | none | | | 0.15 per cent | | | 0.15 per cent | | |
| Treatment of ripened culture | | | p.p.m. | | | p.p.m. | | p.p.m. | | p.p.m. | | | p.p.m. | | | p.p.m. | | | p.p.m. | |
| Holding conditions | Per cent acid added | pH* | $Ac_2$ | $Amc + Ac_2$ | pH | $Ac_2$ | $Amc + Ac_2$ | $Ac_2$ | $Amc + Ac_2$ | $Ac_2$ | $Amc + Ac_2$ | pH | $Ac_2$ | $Amc + Ac_2$ | pH | $Ac_2$ | $Amc + Ac_2$ | pH | $Ac_2$ | $Amc + Ac_2$ |
| Analysed at once | none | 4.55 | 0.36 | 87. | 4.55 | 0.63 | 210. | 0.65 | 87. | 0.89 | 175. | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Held at 70° F. for 9–12 hrs. | none | 4.55 | 0.90 | 70. | 4.55 | 1.30 | 208. | 1.12 | 112. | 1.90 | 250. | 4.50 | 1.15 | 99. | 4.45 | 1.10 | 240. | 4.63 | 0.95 | 140. |
| | citric 0.08 | 4.38 | 2.00 | 186. | 4.36 | 1.95 | 345. | 2.00 | 232. | 3.10 | 410. | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | .15 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 4.31 | 2.00 | 266. | 4.12 | 5.35 | 458. | 4.30 | 2.80 | 332. |
| | .25 | 4.09 | 3.32 | 257. | 4.08 | 3.40 | 515. | 3.53 | 208. | 4.30 | 492. | 4.07 | 3.10 | 250. | ..... | ..... | ..... | ..... | ..... | ..... |
| | .35 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.98 | 4.70 | 210. | 3.89 | 9.60 | 580. | 4.00 | 5.00 | 385. |
| | .45 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.89 | 6.50 | 200. | ..... | ..... | ..... | ..... | ..... | ..... |
| | sulfuric 0.17 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 4.08 | 1.90 | 248. | 4.27 | 2.10 | 182. |
| | .23 | 4.11 | 1.45 | 100. | 4.11 | 1.87 | 313. | 2.50 | 154. | 3.85 | 357. | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | .30 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.85 | 2.00 | 258. | 4.04 | 3.00 | 182. |
| Held in ice for 12–15 hrs. | none | 4.55 | 2.65 | 87. | 4.55 | 3.00 | 238. | 2.65 | 123. | 2.06 | 200. | 4.50 | 3.05 | 120. | 4.45 | 2.60 | 255. | 4.63 | 3.25 | 177. |
| | citric 0.08 | 4.38 | 3.32 | 148. | 4.36 | 4.40 | 285. | 3.70 | 127. | 5.00 | 240. | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | .15 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 4.31 | 4.15 | 137. | 4.12 | 7.00 | 357. | 4.30 | 4.15 | 230. |
| | .25 | 4.09 | 3.95 | 125. | 4.08 | 5.20 | 263. | 3.95 | 114. | 6.40 | 188. | 4.07 | 5.17 | 133. | ..... | ..... | ..... | ..... | ..... | ..... |
| | .35 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.98 | 5.35 | 120. | 3.89 | 8.00 | 345. | 4.00 | 5.65 | 220. |
| | .45 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.87 | 5.55 | 113. | ..... | ..... | ..... | ..... | ..... | ..... |
| | sulfuric 0.17 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 4.08 | 4.70 | 266. | 4.27 | 4.07 | 200. |
| | .23 | 4.11 | 4.15 | 103. | 4.15 | 5.00 | 264. | 5.00 | 114. | 6.00 | 210. | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | .30 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.85 | 5.35 | 250. | 4.04 | 4.85 | 200. |

* pH taken after adding acid to ripened cultures.

(obviously pH optima for acetylmethylcarbinol production since this compound comprises all but a small fraction of the mixture). Apparently there is also a definite optimum pH for production of diacetyl with sulfuric or citric acid which seems to be lower than the pH developed in a normal butter culture. Addition of citric acid to a ripened culture also lowers the pH, but the data indicate its chief rôle is to supply the parent substance for diacetyl and the carbinol.

## DISCUSSION

Various factors tended to give relatively high diacetyl contents in butter cultures. A consideration of these suggests that while a supply of citric acid is important, an oxygen supply also is of significance. Evidently, there is some oxidation reaction involved in the formation of diacetyl, and oxygen also may prevent the reduction of this compound. Since shaking was effective in this connection, the normal agitation of a culture in a butter plant probably has a value other than simply breaking up the curd. The effectiveness of extreme aeration is shown by the work of Brewer *et al.* (1). The acetylmethylcarbinol contents are influenced by the same general factors affecting the diacetyl contents and in this connection oxygen presumably tends to prevent reduction to 2,3-butylene glycol.

Analytical results on successive days for ripened culture held cold show that changes in the diacetyl and the carbinol contents can occur at temperatures considerably below those used for ripening cultures. The addition of citric acid to the cultures held cold tended to give increases in both these products which emphasizes the importance of this acid in the fermentation. These results are in agreement with practices in those plants which regularly hold ripened butter culture cold at least 24 hours before using it in cream.

The pH is a factor influencing the diacetyl and acetylmethylcarbinol contents of cultures. Apparently, the most favorable pH values are lower than the pH range of normal butter cultures, but possibly they vary with temperature.

A high diacetyl content would be assumed to accompany a high acetylmethylcarbinol content but various factors, such as the oxygen supply, temperature, absence of citric acid, etc., may prevent establishment of any close relationship.

## SUMMARY

In studies on the changes in the diacetyl and the acetylmethylcarbinol contents of butter cultures under a variety of conditions, the factors found to favor a high diacetyl content were:

1. Addition of citric acid to the milk used. About 0.15 per cent citric acid appeared to be a practical amount. Larger percentages, such as 0.30

per cent, did not give correspondingly higher diacetyl contents unless the ripened cultures were held cold for an extended period with sufficient aeration.

2. Aeration of the cultures. Shaking the ripened cultures produced significant increases in diacetyl and lack of aeration caused decreases. Under practical conditions, normal agitation undoubtedly gives the same effect.

3. Holding the ripened cultures. There were commonly increases in diacetyl during the holding of cultures at various temperatures, but holding in the cooler is apparently the most practical.

4. Addition of citric acid to the ripened cultures. As little as 0.08 per cent was effective, the agitation incident to the distribution presumably also being a factor.

5. Production of a low pH in the ripened cultures. Lowering the pH of ripened cultures, even with such an acid as sulfuric, tended to increase the diacetyl content.

In general, a high acetylmethylcarbinol content was favored by similar conditions. The attainment of a relatively high content at low temperatures is slower with the carbinol than with diacetyl.

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# CHANGES IN THE DIACETYL AND THE ACETYLMETHYLCARBINOL CONTENTS DURING THE MANUFACTURE OF BUTTER[1]

E. A. PRILL[2] AND B. W. HAMMER

Results of various investigations indicate that diacetyl is an important flavor constituent of butter. It is contributed by the butter culture used and by the cream. Apparently, in the fermentation characteristic of butter cultures the diacetyl is formed through an oxidation of acetylmethylcarbinol which is produced from citric acid, both the production and oxidation being brought about primarily by the citric acid fermenting streptococci (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*) normally present. Sour cream contains diacetyl and acetylmethylcarbinol as a result of bacterial action, but traces of these products also appear to be present in sweet cream which presumably has not undergone any considerable change due to organisms.

Since butter culture and cream contain much less diacetyl than acetylmethylcarbinol, and also because often citric acid is still present, there is an opportunity for changes in the diacetyl and the carbinol contents during the manufacture of butter. Data on this point are presented herein.

## METHODS

### *Manufacturing Procedure*

The butter studied was made during the spring and early summer of 1938. The cream was delivered to the butter laboratory of the Iowa State College from farms in the vicinity, and was made into butter in the usual operation of the laboratory[3] (6).

Both the sweet and sour cream contained approximately 30 per cent fat. The cream was pasteurized at 150° F. for 30 minutes, the sour cream being neutralized to about 0.25 per cent acid prior to pasteurization; following cooling, both the sweet and sour cream were neutralized to approximately 0.10 per cent acid. The churnings in which aerated or modified aerated butter culture was used and some of those in which regular butter culture was employed with sweet cream were made with 150 lb. portions of cream, while the remainder were of commercial size.

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[3] The churnings were made under the supervision of Dr. N. E. Fabricius who is in charge of the butter laboratory.

### *Analytical Procedures*

Several investigators have reported on certain aspects of the changes in diacetyl and acetylmethylcarbinol contents during the manufacture of butter. Barnicoat (1) used his colorimetric method (2) which is based on a comparison of the yellow chloroform solutions of nickel dimethylglyoximate, Mohr and Wellm (9) employed their modification (10) of this procedure, Brioux and Jouis (4) made use of the colorimetric diaminobenzidine method (11), while Fabricius and Hammer (6), Davies (5), Krenn (8), and Virtanen (14) employed some form of the gravimetric procedure. The results reported by these investigators do not permit of close comparisons because of differences in the methods of buttermaking in the various countries and perhaps also because of the different analytical methods used.

In the present work the diacetyl was conveniently determined by the colorimetric procedure recently developed by Prill and Hammer (12) which was designed to avoid the difficulties encountered with some of the other methods, particularly when relatively small amounts of diacetyl are involved. The principle of the method is the conversion of the diacetyl into dimethylglyoxime and then into the intensely colored ammono-ferrous dimethylglyoximate.

The acetylmethylcarbinol plus diacetyl was also determined colorimetrically. Small aliquots of the distillate from the cream or culture distilled with added ferric chloride were used under special conditions (13). Samples of butter (20 g. each) with ferric chloride added were distilled in a special apparatus (12).

The results are recorded as milligrams of diacetyl or of acetylmethylcarbinol plus diacetyl per kilogram of the sample, which is synonymous with expressing them as parts per million.

## RESULTS

In some of the first trials it was noted that the neutralized and pasteurized cream frequently had a lower diacetyl content than the raw cream and, accordingly, a more detailed study of this point was made with several lots of sour cream. The results, which are shown in Table 1, indicate some rather unexpected changes. Shortly after the preliminary neutralization of the cream to 0.25 per cent acid (the original acidity was often 0.5 to 0.6 per cent), or during the first part of the heating, the diacetyl content fell to a very low value and the carbinol content commonly decreased slightly. This may have been due to a reduction of these compounds to 2,3-butylene glycol (7) in the less acid medium. When the cream had been heated to pasteurizing temperature and in the subsequent tests the diacetyl contents showed very significant increases which were probably actually even greater than shown by the analyses because of loss through

TABLE 1

*Changes in diacetyl and acetylmethylcarbinol contents during neutralization and pasteurization of sour cream*

| Cream | Trial 1 | | Trial 2 | | Trial 3 | | Trial 4 | | Trial 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Milligrams per kilogram | | | | | | | | | |
| | $Ac_2$* | Amc† $+Ac_2$ | $Ac_2$ | Amc $+Ac_2$ | $Ac_2$ | Amc $+Ac_2$ | $Ac_2$ | Amc $+Ac_2$ | $Ac_2$ | Amc† $+Ac_2$ |
| Raw | 1.11 | 39.0 | 0.61 | 37.0 | 0.71 | 22.0 | 0.94 | 26.0 | 1.10 | 27.0 |
| Neut. to 0.25% acidity | .24 | 37.0 | .15 | 24.0 | .20 | 26.0 | .30 | 26.0 | .10 | 29.0 |
| Heated to 100° F. | | | | | | | .16 | 23.0 | .12 | 22.0 |
| Heated to 150° F. | | | | | | | .55 | 33.0 | .39 | 27.0 |
| After holding 30 min. | | | | | | | .52 | 32.0 | .43 | 29.0 |
| Cooled | | | | | .48 | 28.0 | .75 | 33.0 | .48 | 30.0 |
| After final neutralization to 0.10% acidity | | | .55 | 36.0 | .56 | 28.0 | .74 | 32.0 | .43 | 29.0 |
| After holding about 1 hr. | .51 | 40.0 | .35 | 32.0 | | | .86 | 32.0 | .57 | 28.0 |

* $Ac_2$ = diacetyl.
† Amc = acetylmethylcarbinol.

volatilization. The carbinol content showed an increase when the cream had reached the pasteurizing temperature and then remained fairly constant in the subsequent tests. The increase in diacetyl may possibly have been caused by non-biological oxidation but the increase in the carbinol through non-biological reactions seems improbable. The net result of the processing of the cream was a decrease in the diacetyl content and relatively little change in the acetylmethylcarbinol content.

Barnicoat (1) reported that during neutralization and pasteurization of cream (presumably the flash method) the carbinol is partly destroyed while the diacetyl is practically unchanged. Apparently, generalizations on these points which are applicable to all methods of processing cream cannot be made at present.

For the churnings used in studying the changes in the diacetyl and acetylmethylcarbinol contents, the butter culture was made with addition of 0.15 per cent citric acid (monohydrate) to the skim or whole milk employed, and from 5 to 10 per cent was added to the pasteurized and cooled cream. Except in one case, which is indicated, the mixture was held cold over night (36° to 44° F. so there would be no significant increase in acid) and churned the next morning. Samples of the processed cream, the butter culture, the mixture of cream and culture immediately after its preparation, the mixture just before churning, the mixture partly churned, the buttermilk, and the finished salted butter were analysed within 1 or 2 hours; they were kept cold during this interval. From the percentages of culture used and on the basis that 30 per cent cream plus 5 to 10 per cent culture yields approximately 1 part of butter (80 per cent fat) and 2 parts of buttermilk, the milligrams of diacetyl and of the carbinol plus

TABLE 2

*Changes in diacetyl and acetylmethylcarbinol contents during manufacture of butter from sweet cream with regular culture added*

| Trial no. | Per cent culture added to cream | Compounds determined | Diacetyl ($Ac_2$) and acetylmethylcarbinol plus diacetyl (Amc + $Ac_2$) in mg. per kg. | | | | | | Estimated per cent of the total $Ac_2$ or Amc + $Ac_2$ retained in butter |
|---|---|---|---|---|---|---|---|---|---|
| | | | Past. neut. cream | Butter culture | Cream plus culture: at once | Cream plus culture: just before churning | Cream plus culture: partly churned | Buttermilk | Butter | |
| 1 | 10 | $Ac_2$ | .03 (*.03*)* | 1.53 (*.15*) | .32 | | | 1.60 (*1.07*) | .33 (*.11*) | |
| | | Amc + $Ac_2$ | 5.1 (*4.6*) | 315. (*31.5*) | 34. | | | 58. (*39.*) | 8.0 (*2.7*) | |
| 2 | 10 | $Ac_2$ | .10 (*.09*) | .69 (*.07*) | .39 | .80 | .82 | 1.33 (*.89*) | .28 (*.09*) | 11.0 |
| | | Amc + $Ac_2$ | 2.8 (*2.5*) | 278. (*28.*) | 27. | 37. | | 46. (*31.*) | 5.5 (*1.8*) | 4.9 |
| 3 | 7 | $Ac_2$ | .08 (*.07*) | .90 (*.06*) | .17 | .26 | .71 | .81 (*.54*) | .14 (*.05*) | 7.0 |
| | | Amc + $Ac_2$ | 3.3 (*3.1*) | 270. (*19.*) | 20. | 21. | 18. | 27. (*18.*) | | |
| 4 | 10 | $Ac_2$ | .08 (*.07*) | .90 (*.09*) | .24 | .50 | .62 | .86 (*.57*) | .18 (*.06*) | 9.7 |
| | | Amc + $Ac_2$ | 3.3 (*3.0*) | 270. (*27.*) | 30. | 30. | | 51. (*34.*) | | |
| 5 | 7 | $Ac_2$ | .18 (*.17*) | .83 (*.06*) | .32 | .56 | .60 | .96 (*.64*) | .18 (*.06*) | 10.0 |
| | | Amc + $Ac_2$ | 7.7 (*7.1*) | 300. (*21.*) | 24. | 26. | | 38. (*24.*) | 4.0 (*1.3*) | 5.1 |
| 6 | 7 | $Ac_2$ | .25 (*.23*) | .52 (*.04*) | .42 | 1.18 | | 1.66 (*1.10*) | .30 (*.10*) | |
| | | Amc + $Ac_2$ | 12.5 (*11.6*) | 232. (*16.*) | 30. | 50. | | 70. (*47.*) | 5.5 (*1.8*) | 3.6 |
| 7 | 10 | $Ac_2$ | .16 (*.14*) | 2.56 (*.26*) | .41 | .54 | .61 | .61 (*.40*) | .18 (*.06*) | 9.8 |
| | | Amc + $Ac_2$ | 7.7 (*6.9*) | 147. (*15.*) | 24. | 25. | | 30. (*20.*) | 3.6 (*1.2*) | 4.8 |
| 8 | 10 | $Ac_2$ | .05 (*.04*) | 3.60 (*.36*) | .47 | .83 | .97 | 1.35 (*.90*) | .21 (*.07*) | 7.2 |
| | | Amc + $Ac_2$ | 2.5 (*2.2*) | 260. (*26.*) | 29. | 36. | | 53. (*35.*) | | |
| 9 | 10 | $Ac_2$ | .09 (*.08*) | 2.16 (*.22*) | .49 | 1.05 | 1.17 | 1.47 (*.97*) | .29 (*.10*) | 8.5 |
| | | Amc + $Ac_2$ | 3.7 (*3.3*) | 306. (*31.*) | 35. | 49. | | 68. (*45.*) | 5.8 (*1.9*) | 3.9 |
| 10 | 10 | $Ac_2$ | .10 (*.09*) | .77 (*.08*) | .40 | 1.05 | 1.10 | 1.66 (*1.10*) | .33 (*.11*) | 10.0 |
| | | Amc + $Ac_2$ | 4.2 (*3.8*) | 360. (*36.*) | 39. | 56. | | 85. (*56.*) | 7.8 (*2.6*) | 4.7 |
| 11 | 10 | $Ac_2$ | .33 (*.30*) | .70 (*.07*) | .87 | 1.37 | 1.55 | 1.97 (*1.31*) | .38 (*.13*) | 8.1 |
| | | Amc + $Ac_2$ | 16. (*14.5*) | 250. (*25.*) | 47. | 60. | 61. | 74. (*49.*) | 7.3 (*2.4*) | 4.0 |
| 12 | 10 | $Ac_2$ | .09 (*.08*) | .56 (*.06*) | .32 | 1.30 | 1.64 | 1.48 (*1.00*) | .36 (*.12*) | 7.3 |
| | | Amc + $Ac_2$ | 3.9 (*3.5*) | 257. (*26.*) | 26. | 53. | | 74. (*49.*) | 7.9 (*2.7*) | 5.1 |

* Numbers in parentheses and italics denote the estimated mg. of $Ac_2$ or of Amc + $Ac_2$ contributed to, or derived from, 1 kg. of the churning mixture by the materials indicated.

diacetyl contributed to, or derived from, 1 kg. of the churning mixture were estimated. The percentages of the diacetyl and of the carbinol plus diacetyl in the churning mixtures that were retained in the butter were also estimated.

Data obtained when regular butter culture was used with sweet cream are given in Table 2. The mixture of cream and culture at once usually contained approximately the amount of acetylmethylcarbinol contributed by the cream and the culture, whereas the diacetyl content was regularly higher than the calculated amount. The incorporation of air during the mixing presumably made it possible for the organisms to effect considerable oxidation. The next morning the diacetyl had increased further, often more than doubling over night, and the carbinol also showed some increases but the percentage increases were not as great as in the case of the diacetyl. During churning there were further increases in diacetyl; the carbinol, however, would not be expected to change greatly in the short period involved.

Fabricius and Hammer (6) found that when mixtures of cream and culture were held for a time before churning, the acetylmethylcarbinol plus diacetyl contents increased and the resulting butter scored higher than butter made by churning soon after the addition of the culture. Other investigators (8, 9, 14) also noted significant increases in the diacetyl and the carbinol contents during holding and churning of cream. Recent work (13) shows that similar increases occur in butter culture under analogous treatments.

The diacetyl and acetylmethylcarbinol contents of the butter and buttermilk are considered later with the values from the other series of churnings.

Table 3 presents results obtained when regular culture was used with sour cream. More diacetyl was contributed to the mixtures of cream and culture by the neutralized and pasteurized cream than by the regular culture, and acetylmethylcarbinol was contributed in comparable amounts from the two sources. However, the culture supplied active organisms which made possible a further accumulation of these two products. Except for a few irregularities, the changes occurring in the diacetyl and acetylmethylcarbinol contents were similar to those obtained with mixtures of sweet cream and regular culture.

Results obtained when aerated (3) or modified aerated cultures were used with sweet cream are given in Table 4. The aerated cultures were grown with aeration under pressure for 12 to 13 hours and had extremely high diacetyl contents (up to 37.4 mg. per kg.); the acetylmethylcarbinol contents were also much higher than those of regular cultures. The modified aerated cultures were aerated under pressure for 3 hours after the usual ripening period; they contained relatively large amounts of diacetyl and the carbinol contents were somewhat higher than those of regular

TABLE 3

*Changes in diacetyl and acetylmethylcarbinol contents during manufacture of butter from sour cream with regular culture added*

| Trial no. | Per cent culture added to cream | Compounds determined | Diacetyl ($Ac_2$) and acetylmethylcarbinol plus diacetyl (Amc + $Ac_2$) in mg. per kg. | | | | | | | Estimated per cent of the total $Ac_2$ or Amc + $Ac_2$ retained in butter |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Past. neut. cream | Butter culture | Cream plus culture | | | Buttermilk | Butter | |
| | | | | | at once | just before churning | partly churned | | | |
| 1 | 5 | $Ac_2$ | .54 (*.52*)* | .94 (*.05*) | .54 | .42 | 1.00 | 1.25 (*.84*) | .20 (*.07*) | 6.7 |
| | | Amc + $Ac_2$ | 7.7 (*7.3*) | 250. (*13.*) | 27. | 39. | | 55. (*37.*) | 6.1 (*2.1*) | 5.3 |
| 2† | 10 | $Ac_2$ | .15 (*.14*) | .65 (*.07*) | .70 | .79 | | 1.18 (*.79*) | .37 (*.12*) | |
| | | Amc + $Ac_2$ | 9.6 (*8.7*) | 305. (*31.*) | 47. | 55. | | 60. (*40.*) | 5.5 (*1.8*) | 3.3 |
| 3 | 5 | $Ac_2$ | .43 (*.41*) | .33 (*.02*) | .47 | 1.13 | 1.20 | 1.20 (*.80*) | .32 (*.11*) | 8.9 |
| | | Amc + $Ac_2$ | 20.7 (*19.6*) | 230. (*12.*) | 38. | 42. | 40. | 52. (*35.5*) | 5.3 (*1.8*) | 4.5 |
| 4 | 5 | $Ac_2$ | .36 (*.34*) | | .38 | .36 | .43 | .55 (*.37*) | .16 (*.05*) | 12.3 |
| | | Amc + $Ac_2$ | 14.5 (*13.8*) | | 20. | 17. | 15. | 21. (*14.*) | 2.8 (*.9*) | 6.2 |
| 5 | 5 | $Ac_2$ | .40 (*.38*) | .38 (*.02*) | .52 | .51 | 1.05 | 1.22 (*.82*) | .27 (*.09*) | 8.5 |
| | | Amc + $Ac_2$ | 19. (*18.*) | 266. (*13.*) | 30. | 46. | | 74. (*50.*) | 5.0 (*1.7*) | 3.7 |
| 6 | 5 | $Ac_2$ | .47 (*.45*) | .59 (*.03*) | .46 | 1.00 | | 1.07 (*.72*) | .36 (*.12*) | |
| | | Amc + $Ac_2$ | 28. (*27.*) | 270. (*14.*) | 39.5 | 53. | | 74. (*50.*) | 6.5 (*3.2*) | 6.0 |
| 7 | 7 | $Ac_2$ | .51 (*.47*) | .34 (*.03*) | .46 | 1.10 | 1.08 | 1.10 (*.73*) | .40 (*.13*) | 12.0 |
| | | Amc + $Ac_2$ | 40.5 (*37.*) | 262. (*18.*) | 51. | 60. | | 79. (*53.*) | 7.1 (*2.4*) | 4.0 |
| 8 | 8 | $Ac_2$ | .35 (*.32*) | .33 (*.03*) | .66 | | 1.00 | 1.10 (*.73*) | .25 (*.08*) | 8.3 |
| | | Amc + $Ac_2$ | 32. (*29.*) | 130. (*10.*) | 40. | | 41. | 55. (*37.*) | 4.4 (*1.5*) | 3.6 |

* See footnote of Table 2.

† In this trial cream was prepared and churned the same day.

cultures. Analyses of the mixtures of cream and culture at once do not indicate an increase in diacetyl during mixing but rather some of them suggest a slight decrease, especially in the case of the aerated cultures. It appears that after having grown in a strongly aerated medium the organisms did not immediately effect further oxidations leading to production of diacetyl on transfer to the less aerated medium. On holding until the next morning and during churning, the organisms usually produced additional diacetyl and acetylmethylcarbinol and although, in most cases, the actual increment in diacetyl was as great (or greater) as with regular culture, the percentage increase was not, because of the relatively large amount of diacetyl originally present. Consequently, in comparisons involving the addition of equal percentages of regular and aerated or modified aerated culture to pasteurized sweet cream, the butter made with the latter types of culture invariably had the higher diacetyl content (0.38 to 1.00 mg. per kg. as compared to 0.14 to 0.38 mg. per kg.), but these higher values were really much lower than they would be if the diacetyl contents of the butter were proportional to those of the cultures used. In case the cream is churned soon after the addition of the culture, however, aerated or modified aerated cultures probably would show a greater practical advantage over regular cultures.

In the distribution of the diacetyl and the acetylmethylcarbinol between the butter and the buttermilk, certain general relationships apply to all the churnings studied. The estimated milligrams of diacetyl in the buttermilk and the butter derived from 1 kg. of the churning mixture agree fairly well, in most cases, with the diacetyl content of the partly churned mixture. Also, the estimated milligrams of the carbinol plus diacetyl in the two products agree fairly well with the content just before churning. Except for a few irregularities, the diacetyl contents of the butter were very nearly 1/5 of the diacetyl contents of the corresponding buttermilk. Since butter contains about 1/5 serum it would appear at first sight that the diacetyl is contained entirely in the serum of the butter. However, the carbinol plus diacetyl content of the butter was, in most cases, only about 1/10 that of the buttermilk, which is much less than would be expected because acetylmethylcarbinol is presumably even more preferentially dissolved in the water phase than is the diacetyl. The average of the estimated percentages of the diacetyl in the churning mixtures that were retained in the butter was 8.8 per cent, and the corresponding value for the carbinol plus diacetyl was 4.5 per cent.

Barnicoat (1) reported that 0 to 33 per cent (average 15 per cent) of the diacetyl and 7 to 22 per cent (average 14 per cent) of the carbinol in cream were retained in the butter, and on the basis of the averages of these divergent values postulated that diacetyl and the carbinol were contained in the butter serum. Actually, a number of his analyses of individual churnings

TABLE 4

*Changes in diacetyl and acetylmethylcarbinol contents during manufacture of butter from sweet cream with aerated or modified aerated culture added*

| Trial no. | Per cent and type culture added to cream | Compounds determined | Diacetyl ($Ac_2$) and acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) in mg. per kg. | | | | | | | Estimated per cent of the total $Ac_2$ or $Amc + Ac_2$ retained in butter |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Past. neut. cream | Butter culture | Cream plus culture | | | Buttermilk | Butter | |
| | | | | | at once | just before churning | partly churned | | | |
| 1 | 10 a† | $Ac_2$ | .10 (.09)* | 16.8 (1.68) | 1.55 | 1.39 | 2.22 | 2.44 (1.65) | .40 (.13) | 6.0 |
| | | $Amc + Ac_2$ | 2.8 (2.5) | 410. (41.) | 36. | 34. | | 47. (31.) | 4.8 (1.6) | 4.7 |
| 2 | 10 a | $Ac_2$ | .08 (.07) | 25.0 (2.50) | 2.30 | 2.40 | 2.90 | 3.60 (2.40) | .60 (.20) | 6.9 |
| | | $Amc + Ac_2$ | 3.3 (3.0) | 570. (57.) | 54. | 68. | 68. | 69. (46.) | | |
| 3 | 10 a | $Ac_2$ | .16 (.14) | 26.8 (2.68) | 2.60 | 3.25 | 3.70 | 3.90 (2.60) | 1.00 (.33) | 9.0 |
| | | $Amc + Ac_2$ | 7.7 (6.9) | 750. (75.) | | 90. | | 118. (79.) | 13.4 (4.5) | 5.0 |
| 4 | 10 a | $Ac_2$ | .05 (.04) | 37.4 (3.74) | 3.85 | 3.85 | 3.35 | 5.30 (3.52) | .93 (.31) | 9.3 |
| | | $Amc + Ac_2$ | 2.5 (2.2) | 920. (92.) | 85. | 97. | | 120. (80.) | | |
| 5 | 10 ma‡ | $Ac_2$ | .09 (.08) | 12.7 (1.27) | 1.43 | 1.58 | 1.43 | 2.31 (1.54) | .44 (.15) | 10.2 |
| | | $Amc + Ac_2$ | 3.7 (3.3) | 410. (41.) | 43. | 46. | | 72. (48.) | 6.5 (2.2) | 4.8 |
| 6 | 10 ma | $Ac_2$ | .10 (.09) | 10.0 (1.00) | .91 | 1.14 | 1.40 | 2.00 (1.33) | .38 (.13) | 9.0 |
| | | $Amc + Ac_2$ | 4.2 (3.8) | 308. (31.) | 35. | 40. | | 59. (39.) | 5.6 (1.8) | 4.7 |
| 7 | 10 ma | $Ac_2$ | .33 (.30) | 15.5 (1.55) | 1.96 | 2.60 | 2.72 | 3.90 (2.60) | .68 (.23) | 8.5 |
| | | $Amc + Ac_2$ | 16.0 (14.5) | 417. (42.) | 53. | 75. | 74. | 98. (65.) | 9.3 (3.1) | 4.2 |
| 8 | 10 ma | $Ac_2$ | .09 (.08) | 17.5 (1.75) | 1.47 | 2.22 | 2.70 | 2.72 (1.81) | .56 (.19) | 6.9 |
| | | $Amc + Ac_2$ | 3.9 (3.5) | 323. (32.) | 37. | 62. | 69. | 82. (55.) | 6.8 (2.3) | 3.3 |

* See footnote of Table 2.
† a = aerated butter culture.
‡ ma = modified aerated butter culture.

showed a ratio of approximately 2 to 1 for the percentages of diacetyl and of the carbinol retained in the butter. The results obtained by Mohr and Wellm (9) indicate a lower percentage retention of diacetyl and a still lower percentage retention of the carbinol. They attributed this to the removal of some of these products by the wash water, presumably, relatively more of the carbinol than diacetyl being washed out. Krenn (8) made similar claims in regard to the washing. In the present work the analyses of a few samples of wash water showed only fractions of a milligram of the carbinol plus diacetyl per kilogram. While the total amount present in the wash water may be significant in comparison with the amount present in the butter, it is difficult to differentiate between material which comes from the film of buttermilk remaining between the butter granules and that which might possibly be removed from the granules themselves. If the diacetyl and the carbinol are both held entirely in the serum droplets in the same manner, they should be retained in the butter in the same proportion as they exist in the churning mixture at the time the butter is formed, and any possible washing out of these products should also be in this proportion. Actually, the distribution of the diacetyl and the carbinol in butter is probably more complex than has been assumed. The serum in the form of minute droplets may possibly have solvent properties different from those of larger volumes of this fluid. The possibility of the adsorption of the diacetyl at the enormous water-fat interface should also be considered, as well as the possibility of the solution of a portion of the diacetyl in the fat.

With the churnings made by adding any of the types of culture to sweet cream it was not possible to note a close relationship between the diacetyl contents of the culture and of the finished butter. Undoubtedly other factors, such as acetylmethylcarbinol content, acidity, oxygen tension, and numbers and activity of the organisms present are also involved. It would be even more difficult to detect a relationship when sour cream is used because of the greater variation in the quality of such cream and the comparatively large amount of diacetyl it contributes to the mixture of cream and culture.

## SUMMARY

Marked changes in diacetyl content, both decreases and increases, were noted during the neutralization and pasteurization of sour cream. The changes in the acetylmethylcarbinol content were relatively small.

Progressive increases in diacetyl were found after mixing regular butter culture with pasteurized cream (either sweet or sour), after holding the mixture at a low temperature over night, and after the mixture had been partly churned. The acetylmethylcarbinol content increased somewhat during the holding period. The results when aerated or modified aerated butter culture was added to sweet cream were similar to those when regular culture was employed, except that no increases in diacetyl were noted

immediately after mixing, and the subsequent increases, while quite significant in actual amounts, were small on a percentage basis because of the relatively large amounts of diacetyl already supplied by these cultures.

It was not possible to note a close relationship between the diacetyl contents of the butter cultures added to the cream and those of the resulting butter. Aerated or modified aerated cultures, as compared with equal percentages of regular cultures, yielded butter having higher diacetyl contents, but these values were really much lower than they would have been if a proportionality existed between the diacetyl contents of the cultures and the butter.

On the average, 8.8 per cent of the diacetyl and 4.5 per cent of the acetylmethylcarbinol plus diacetyl in the churning mixture were retained in the butter.

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# EFFECTS OF THE COLD STORAGE TEMPERATURE, HEAT TREATMENT, AND HOMOGENIZATION PRESSURE ON THE PROPERTIES OF FROZEN CONDENSED MILK

R. W. BELL

*Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture*

The effects of freezing on whole milk and condensed milk have been studied by numerous investigators. Webb and Hall (1) studied primarily the physical effects of freezing and storage, and suggested the use of frozen condensed milk as a source of fresh market milk where the latter is expensive or not available. More recent studies of the freezing of milk and milk products have been made by Baldwin and Doan (2), Doan and Baldwin (3), Doan and Featherman (4), and Heiss (5).

The work of Webb and Hall has been extended in order to ascertain more in detail the desirable features of the process for preparing and preserving frozen condensed milk. Concentration milks of not more than 8.0 per cent fat content were prepared, and the factors investigated were those which must be considered in the commercial development of frozen condensed milk.

## EFFECT OF STORAGE TEMPERATURE

The milk was produced by the Bureau of Dairy Industry herd at Beltsville, Maryland, and handled in the dairy plant there. There was opportunity for it to dissolve copper from equipment with which it came in contact. In this experiment the milk contained 3.3 per cent fat and 12.9 per cent solids. It was homogenized at the dairy at 2600 pounds pressure and a temperature of 55° C. (131° F.). The flavor score on delivery in Washington was 23.0 and the viscosity 3.8 centipoises at 20° C. (68° F.). A flavor score of 23 is considered excellent; 21 to 23 is good, and 18 to 21 fair. A flavor score of less than 18 indicates poor or bad milk. Data on flavor indicate the opinion of C. J. Babcock, market milk specialist of the Bureau, without whose assistance it would not have been possible to so accurately judge this property.

Pasteurization of the 5 gallons of homogenized milk was carried out in 3 porcelain-lined containers set in a steam bath, by heating quickly with some agitation to 68° C. (154.4° F.) and maintaining the temperature for 30 minutes. At the end of this period the milk was drawn into 3 glass balloon flasks in 2 to 3 minutes and concentrated under a vacuum of 26 to 27 inches to 6.5 per cent fat and 25.3 per cent solids. The flavor score of the reconstituted milk was 22.5; the criticism, flat. The viscosity of the con-

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TABLE 1–A AND 1–B

*Comparison of 3 cold storage temperatures on properties of condensed milk*

1–A. Whole milk homogenized at 2600 pounds pressure and 55° C.; pasteurized by heating to 68° C. and holding for 30 minutes

| Weeks in storage | (a) Storage temperature 0° C. | | | | (b) Storage temperature – 7° C. | | | | (c) Storage temperature – 17° C. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flavor | Viscosity | Body | Bacteria per cc. | Flavor | Viscosity | Body | Bacteria per cc. | Flavor | Viscosity | Body | Bacteria per cc. |
| | | *Centipoises* | | | | *Centipoises* | | | | *Centipoises* | | |
| 1 | 22.0 Slightly flat | 8.6 | Good | 600 | 21.5 Flat | 8.0 | Good | 100 | 22.0 Sl. flat | 7.6 | Good | 900 |
| 2 | 21.5 Flat | 8.6 | " | 20,100 | 22.0 Sl. flat | 8.0 | Flakes on bottom of can | 200 | 21.0 Very sl. bitter | 7.7 | Less flaky than (b) | 800 |
| 3 | 19.0 " | 9.2 | " | 600,000 | 19.0 Sl. oxidized | 8.8 | Objectionable curd formation | < 100 | 19.0 Sl. oxidized | 8.2 | Bottom layer and flakes dissolved on pouring | < 100 |
| 4 | Sour | 9.5 | Some fat separation | 14,400,000 | 10.0 Oxidized | 8.9 | Large lumps curdled | ..... | 10.0 Oxidized | 8.7 | Lumpy but better than (b) | ..... |
| 5 | ..... ..... | ..... | ..... | ..... | 15.0 " | 11.0 | " " | < 100 | 15.0 " | 10.2 | " " " | < 100 |

1–B. Condensed milk homogenized at 2300 pounds pressure and 54° C.; pasteurized by heating to 61° C. and holding for 30 minutes

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20.5 Flat | 7.9 | Good | ..... | 21.0 Flat | 7.4 | Some fat separation, sl. flaky | ..... | 21.0 Flat | 7.2 | Thin, semisolid layer on bottom of can | ..... |
| 2 | 19.5 Sl. foreign | 8.3 | " | ..... | 21.0 " | 8.0 | " " | ..... | 21.0 " | 7.2 | " " " Better than (b) | |
| 3 | 18.0 Sl. puckery | 9.0 | White, watery | ..... | 20.0 Sl. foreign | 8.5 | Some fat separation, curdy lumps on bottom of can | ..... | 19.0 Sl. rancid | 7.6 | Some thickening. Better than (b) | ..... |
| 4 | 15.0 Rancid | 9.2 | White, watery, poor | ..... | 19.5 Sl. bitter | 8.6 | Curdy lumps larger and firmer than (c) | ..... | 17.0 Rancid | 8.4 | Flakes on bottom of can | ..... |

centrated product was 6.9 centipoises and the bacterial count 600[1]—a reduction from 3100 per cc. A milk powder-tomato juice medium was used in determining the number of bacteria in the samples. The plates were incubated at 37° C. (98.6° F.).

The condensed milk was cooled to 10° C. (50° F.) and packaged in hermetically-sealed lacquered cans of 140 cc. capacity. The cans were then divided into 3 lots. One lot was stored at 0° C. (32° F.), another at –7° C. (19.4° F.), and the third at –17° C. (1.4° F.). The effect of the storage temperature on the stability of the condensed milk is recorded in Part 1–A of Table 1–A and 1–B.

*Flavor.*—The flavor scores of the samples stored at 0° C. decreased at about the same rate during the first 3 weeks as did the scores of those stored at –7° and –17° C. but for a different reason. An increase in the bacterial count was noted at the end of the second week. By the end of the fourth week the milk was sour. In other experiments quite comparable to this one the rate of increase in the bacterial count was not as rapid and there was evidence that proteolytic organisms predominated. Evidence is ample that bacteria are not quiescent at 0° C. (6) (7).

The flavor scores of the samples stored at –7° C. followed much the same course as did the scores of those stored at –17° C. and for the same reasons. It will be noted that the sample held at –17° C. for 2 weeks was very slightly bitter and was rated 1.0 less than the sample held at –7° C. for the same time. A bitter flavor often precedes an oxidized flavor. Therefore, the evidence here is that, when other conditions are comparable and equally favorable to the development of an oxidized flavor, condensed milk stored at –17° C. develops an oxidized flavor sooner than that held at –7° C. This was corroborated many times in the course of the investigation (See Table 3). If this effect was due to an enzyme which Kende (8) calls oleinase, then we have an unusual example in which an enzyme is apparently more active at –17° C. than at –7° C. In these experiments the rate of freezing was more rapid in the samples stored at –17° C. than in those stored at –7° C., and the latter samples thawed sooner than did those held at the lower temperature when they were placed in the + 6° C. (42.8° F.) incubator where they remained for about 17 hours. But, except for the differences in the rate of freezing and the rate of thawing, the experimental procedure was designed to eliminate variables other than the storage temperature.

The oxidized flavor was less pronounced at 5 weeks than at 4 weeks. It reached a maximum intensity (low numerical score) and then became less pronounced (high numerical score). This will be noted in Tables 3 and 4–A.

[1] Thanks are due F. R. Evans and M. Rogosa for obtaining the bacterial counts.

*Viscosity.*—The viscosity values in centipoises are relatively small. They were obtained at 20° C. They increased slowly as the samples became older and less rapidly the lower the storage temperature.

*Body.*—Continued storage in a frozen condition injures the body of condensed milk. If bacterial growth and enzymatic action did not take place at 0° C. it would be better, so far as body is concerned, to preserve homogenized condensed milk without freezing. While the viscosity values obtained subsequent to tempering the samples stored at 0° C. increased faster than did those of the samples stored at –7 and –17° C. subsequent to thawing and tempering, the differences were not sufficient to decrease the quality of the condensed milk from a commercial standpoint.

The body of the samples stored at –17° C. was superior to the body of the samples stored at –7° C. However, in this and other experiments the advantage was not pronounced and, in general, not sufficient to offset the flavor advantages of storage at –7° C.

A batch was made in which metallic contamination was avoided and the heat treatments were relatively mild and in a different sequence.

The milk was produced in the morning and was hand drawn into well-tinned pails. It contained 4.6 per cent fat and 14.3 per cent total solids. The flavor score was 22.5; the criticism, cowy. The viscosity was 3.76 centipoises. In the Bureau's Washington laboratory the milk was drawn directly into glass balloon flasks and sufficient water vaporized to yield milk of 8.0 per cent fat and 26.1 per cent total solids content. This condensed milk was taken to the Beltsville plant, warmed quickly to 54° C. (129.2° F.), and immediately homogenized at 2300 pounds pressure. The condensed and homogenized milk was then returned to Washington and canned in the small tins and pasteurized. The condensed milk was pasteurized in the following manner: The filled and closed cans were placed in metal trays and the trays slid into the places provided in the cage of a pilot sterilizer. The cage rotated in hot water at the rate of 5 revolutions per minute. Under the conditions indicated above it was found that the contents of the cans were at the temperature of the heating medium in 5, 6, 7, and 8 minutes when the water was at 61°, 71°, 76.5°, and 82° C. (141.8°, 159.8°, 169.7° and 179.6° F.), respectively. At the end of the heating period the cans were dumped into cold running water where their contents cooled quickly.

In this experiment the holding temperature was 61° C. and the holding period 30 minutes. The viscosity at the end of this step and subsequent to tempering at 20° C. was found to be 7.2 centipoises. The flavor score of the reconstituted sample was 22.5; the criticism, slightly sweet.

The results are recorded in part 1–B of Table 1–A and 1–B.

*Flavor.*—Samples stored at 0° C. were inferior in flavor after the first week to those stored at –7° C. and –17° C. After 3 and 4 weeks, samples stored at – 7° C. were superior in flavor to those stored at – 17° C.

The effect of incomplete destruction of the enzyme lipase during processing on the flavor of the stored samples is shown in the results of this experiment. In the presence of lipase that survived the heat incident to processing, the samples, after 2 weeks in storage, became sufficiently hydrolyzed to taste rancid and, unlike the oxidized or tallowy flavor of other batches, the intensity of this flavor continued to increase.

It is not known whether additional experiments would indicate that a rancid flavor becomes more pronounced at –17° C. than at –7° C. This experiment serves to emphasize the importance of destroying lipase before it has had opportunity to adversely affect the fat.

In a commercial operation the properties of the milk and the condensing conditions might be such as to result in a rancid product at the completion of this operation if the lipase in the milk was not destroyed by forewarming.

The lipase should be destroyed before either condensing or homogenizing. According to Dorner and Widmer (9) and Doan (10) this can be accomplished by holding the milk at 60° C. (140° F.) for 10 minutes or by subjecting it to a heat treatment of at least equal enzyme destroying effect. However, if it is assumed that the rancidity noted under the different conditions of the experiments reported here was due to undestroyed lipases, even more severe heat treatments failed to completely destroy the enzyme.

*Viscosity.*—The increase in viscosity was slower the lower the storage temperature. The initial values were low and some fat separation took place in samples that had been stored at – 7° C.

*Body.*—In the first 2 weeks the body of the samples stored at 0° C. was satisfactory. There was no fat separation, the product was fluid and did not adhere to the container. But at 3 weeks a change to a whiter, more watery appearance had taken place. This condition was more pronounced at 4 weeks.

The body of the samples stored at –17° C. was slightly superior to the body of the samples stored at –7° C.

### EFFECT OF PASTEURIZATION METHODS

It is well known that the effect of high temperatures on the properties of milk and its products is marked. In order to obtain information on this subject, in its relation to frozen condensed milk, experiments were designed to learn whether the heating required to check the development of an oxidized flavor causes a cooked flavor. Another purpose was to study the effect of these heat treatments on the viscosity and the body of the samples.

In the making of evaporated milk the high temperature of sterilization prevents the development of an oxidized flavor and increases the viscosity.

The milk was obtained through the regular channels. It was scored 21.5, cowy, and contained 4.4 per cent fat. The fat content of the con-

densed material was 8.0 per cent. Immediately after the heat treatments indicated in Table 2 the contents of the cans were cooled quickly to 10° C. in cold running water and then slow frozen in still air at –7° C.

The (a) and (b) samples were inferior to the others because of fat separation and less desirable flavor. The (c) samples had a slightly better flavor but the body was still objectionable. The (d) samples, that is, those pasteurized by revolving them in water at 76.5° C. for 15 minutes had flavor scores and body properties that were, on the whole, superior to those of the other samples in this batch.

It will be noted that the viscosity of the samples increased with an increase in the pasteurization temperature and in the storage period. The homogenization temperature and pressure were favorable to a low viscosity in the samples. The homogenization pressure in this experiment was insufficient to prevent separation of the fat during slow freezing and thawing in the media of relatively low viscosity.

While pasteurization by exposing milk in the small cans to a temperature of 76.5° C. for 15 minutes was found not to be sufficient in all cases (see Table 3) to prevent the development of an oxidized flavor during a period of 4 weeks, higher heat treatment caused objectionable body changes during storage. Consequently, in so far as the effect of heat during pasteurization is concerned this work indicates that the equivalent of the heat treatment which these (d) samples received ought not to be exceeded in the production of frozen condensed milk that is not to be utilized within a relatively short period. If this heat treatment is exceeded the effect is too detrimental to the body of the product; if it is milder, then oxidative and other changes will be more apt to take place. A balance must be sought in accordance with the experiences of the manufacturer of the product.

None of the samples listed under (a), (b), and (c) were scored "cooked" subsequent to dilution. The (d), (e), and (f) samples tasted slightly cooked or cooked when 1 week old. However, this flavor was not apparent in the samples examined after 2 or more weeks storage.

In Table 3 are presented data on the effect of both pasteurization methods and storage temperatures. A portion of the regular milk supply was used. It contained 3.7 per cent fat and had a flavor score of 23. After condensing to a fat content of 8.0 per cent, the product was heated to 70° C. and homogenized at 1400 pounds pressure. The samples stored at –7° C. solidified in 24 hours; those stored at –17° C. in 6 hours.

From a flavor standpoint the samples stored at –7° C. were preserved better than those held at – 17° C.

The creamy body of samples stored at –7° C. was preferable to the curdy condition of the older –17° C. samples. The curdy lumps were apt to be hard and surrounded by a watery phase. In this experiment the body of

TABLE 2

*Effect of pasteurization methods on properties of condensed milk stored at −7° C.*

| Weeks in storage | (a) Pasteurized by heating for 35 minutes in water at 61° C. | | | (b) Pasteurized by heating for 15 minutes in water at 71° C. | | | (c) Pasteurized by heating for 15 minutes in water at 74° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Condensed milk homogenized at 1000 pounds pressure and 61° C. | | | | | | | | |
| | Flavor | Viscosity | Body | Flavor | Viscosity | Body | Flavor | Viscosity | Body |
| | | *Centipoises* | | | *Centipoises* | | | *Centipoises* | |
| 0 | 21.5 Flat | 6.5 | Good | 21.5 Flat | 6.7 | Good | 22.0 Sl. flat | 6.7 | Good |
| 1 | 21.5 " | 7.7 | Churning effect objectionable | 20.5 Sl. bitter | 7.9 | Same as (a) but less objectionable | 21.5 Flat | 8.0 | Same as (b) but less objectionable |
| 2 | 21.0 Trifle bitter, Sl. flat | 7.8 | " " More than last week | 21.0 Sl. bitter Tr. flat | 7.8 | " " | 21.5 " | 8.4 | " " |
| 3 | 19.0 Sl. bitter | 8.2 | Churned fat on top, curdy lumps in bottom portion | 20.0 Flat, Sl. off | 9.1 | Objectionable fat layer of flaky, churned lumps | 20.5 " | 9.3 | Same as (b) |
| 4 | 19.5 " " | 9.0 | Fat layer on surface. Large lumps in bottom | 20.0 " " | 10.1 | Same as (a) except fewer lumps in bottom | 20.0 " Sl. off | 10.1 | Fat separation about same as (b). No lumps on bottom |
| 5 | 19.0 " " | 10.1 | Fat separation. Whiter fluid in bottom | 19.0 Sl. bitter | 11.3 | Fat separation. Cultured buttermilk condition | 20.5 Flat | 13.1 | Fat layer greater than (d). Thickening in bottom portion |
| | (d) Pasteurized by heating for 15 minutes in water at 76.5° C. | | | (e) Pasteurized by heating for 15 minutes in water at 79.5° C. | | | (f) Pasteurized by heating for 15 minutes in water at 82° C. | | |
| 0 | 22.0 Sl. flat | 7.2 | Good | 20.0 Cooked | 7.6 | Good | 19.0 Cooked | 8.4 | Good |
| 1 | 21.5 Sl. cooked | 9.1 | Good. No fat separation | 21.5 Sl. cooked | 9.8 | Good. No fat separation | 20.0 " | 11.5 | Good. No fat separation |
| 2 | 21.5 Flat | 9.5 | Sl. flaky. Good consistency | 21.5 Flat | 10.6 | Sl. flaky | 21.0 Flat | 15.7 | Same as (e) |
| 3 | 21.5 " | 11.9 | Less fat separation and better than (a), (b), or (c) | 21.0 " | 15.7 | Thickening in can. Creamy buttermilk condition | 21.0 " | 22.8 | No fat separation. Sl. better than (e) |
| 4 | 21.0 " | 11.1 | Fat separation less than (c). Thickening on bottom | 21.0 " | 20.0 | Less fat separation but more thickening than (d) | 21.0 " | 34.5 | Fat separation slight. Creamy thickening. Watery in bottom portion |
| 5 | 21.0 " | 14.2 | Fat layer larger than (e). Sl. flaky | 20.5 " | 26.8 | Thin fat layer, about same as (f), flaky | 20.5 " | 38.8 | Flaky, watery in bottom portion |

TABLE 3

*Effect of pasteurization methods and storage temperatures on properties of condensed milk*

Condensed milk homogenized at 1400 pounds pressure and 70° C.

Stored at – 7° C.

| Weeks in storage | (a) pasteurized by heating for 15 minutes in water at 71° C. | | | (b) Pasteurized by heating for 15 minutes in water at 76.5° C. | | | (c) Pasteurized by heating for 15 minutes in water at 82° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Flavor | Viscosity | Body | Flavor | Viscosity | Body | Flavor | Viscosity | Body |
| | | *Centipoises* | | | *Centipoises* | | | *Centipoises* | |
| 0 | 22.0 Flat | 7.8 | Good | 22.0 Flat | 8.8 | Good | 20.0 Cooked | 12.5 | Good |
| 1 | 20.5 Sl. oxidized | 7.8 | Sl. flaky, otherwise good | 20.5 Sl. oxidized | 10.1 | " | 21.0 Sl. cooked | 20.1 | " |
| 2 | 15.0 Oxidized | 9.1 | Sl. fat separation and flakiness | 15.0 Oxidized | 10.9 | No flakes or fat separation. Sl. thickening in bottom portion | 21.0 " | 21.4 | Same as (b) |
| 3 | 15.0 " | 9.2 | Sl. fat separation and flakiness. A few lumps | 17.0 " | 11.6 | Sl. flaky | 21.0 Flat | 25.1 | Same as (b) but more viscous |
| 4 | 18.0 " | 10.3 | Flaky and curdy particles. No fat separation | 19.0 Sl. oxidized | 17.2 | Creamy. Precipitate on bottom of can | 21.0 " | 43.0 | Thick, creamy, cultured buttermilk body. No fat separation. Objectionable |
| 5 | 19.0 Sl. oxidized | 16.4 | Curdling and lumps in bottom of can | 18.0 Oxidized | 22.5 | Creamy but not flaky | 20.0 Old | 51.3 | " " " |

Stored at – 17° C.

| | (d) Pasteurized as in (a) | | | (e) Pasteurized as in (b) | | | (f) Pasteurized as in (c) | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 15.0 Oxidized | 8.8 | Sl. flaky, otherwise good | 17.0 Oxidized | 10.0 | Same as (d) | 20.5 Cooked | 16.1 | Same as (d) except sl. flaky in bottom portion |
| 2 | 15.0 " | 9.8 | " " No fat separation | 18.0 " | 10.1 | " " " | 21.0 Sl. cooked | 16.5 | Sl. better than (e) |
| 3 | 15.0 " | 9.6 | Good, except precipitate on bottom of can | 17.0 " | 10.7 | No flakes, no fat separation. Same as (d) | 21.0 " | 17.9 | Thickening in bottom portion |
| 4 | 18.0 " | 9.9 | Lumpy and some thickening in bottom portion | 18.0 " | 12.0 | More thickening in bottom portion than (d). Lumps about same | 19.0 Sl. bitter | 29.2 | Curdy with small flakes |
| 5 | 17.0 " | 13.0 | Curdy with watery condition between lumps | 19.0 Sl. oxidized | 17.9 | Inferior to (d) | 18.0 Oxidized | 43.0 | Thicker than (d). Large curdy lumps |

the samples stored at –7° C. was superior to that of samples stored at –17° C.

### EFFECT OF HOMOGENIZATION

In the making of evaporated milk, homogenization is one of the processing steps. Proper homogenization results in a product of homogenous character with respect to the dispersion of the fat phase; consequently a cream layer usually does not form. Freezing and thawing are injurious to the fat emulsion in both whole and condensed unhomogenized milk. This undesirable effect can be eliminated by dispersion of the fat by means of homogenization. Another but less effective way is to increase the viscosity of the condensed milk through vaporization of water until a solids content of 3 to 4 times that of the whole milk has been attained.

It has been shown by Tracy, Ramsey, and Ruehe (11), Thurston, Brown, and Dustman (12), and Ross (13) that homogenization has a marked effect in reducing or in eliminating the susceptibility of milk to oxidized flavor development.

The effect of homogenization on the properties of frozen condensed milk is shown by data collected in Tables 4–A and 4–B. These 2 batches of milk were obtained at an interval of 1 week and were a part of the general milk supply. The condensed milk contained 8.0 per cent fat.

No explanation is attempted as to why homogenization caused the condensed milk to be more resistant to the changes which result in an oxidized flavor. Ross (13) has a hypothesis for whole milk which is based on the assumption that "oxidized flavor is caused by enzymic action on fat globules. If this is true it seems possible that sufficient homogenization pressures surround the finely divided fat globules with a film. This covering protects the fat globules from enzymic action and thus prevents the development of oxidized flavor." In this connection it is interesting to note that Dorner and Widmer (9) in answering the question: "Why did milk become rancid more rapidly after homogenization?" wrote "The effect of homogenization is to reduce the size of the fat globules. A reduction of the size of globules, while the total volume is kept unchanged, produces an increase of the surface. In fact, the surface of the fat is increased about 10 to 30 times through homogenization. The surface of the fat being greater in homogenized milk, the lipase can attack it better." The important point would seem to be not whether more protein is adsorbed by all the fat, but whether each fat globule is better protected by a thicker or more continuous envelope. With a 10 to 30-fold increase in the surface of the fat globules much more protein would have to be adsorbed on the same volume of fat to obtain a protective envelope of as great thickness as before homogenization.

Greenbank (14) reports a more rapid development of an oxidized flavor in market milk the lower the storage temperature within the range 20° to

TABLE 4–A AND 4–B

*Effect of Homogenization Pressure on Properties of Condensed Milk Stored at – 17° C.*

4–A. Condensed milk homogenized at 55° C. and pasteurized by heating to 61° C. and holding for 30 minutes

| Weeks in storage | (a) Unhomogenized | | | (b) Homogenization pressure 1000 pounds | | |
|---|---|---|---|---|---|---|
| | Flavor | Viscosity | Body | Flavor | Viscosity | Body |
| | | *Centipoises* | | | *Centipoises* | |
| 0 | 20.0 Flat | 5.7 | Fat separation and flaky | 21.5 Flat | 6.0 | Better than (a) |
| 1 | 19.0 " | 6.0 | Objectionable cream layer | 19.0 " | 6.5 | Thin deposit on bottom of can |
| 2 | 18.0 Oxidized | 6.1 | " " Bottom portion watery | 19.0 Sl. oxidized | 6.5 | Some fat separation |
| 3 | 10.0 Rancid | 6.5 | " " | 12.0 Oxidized | 7.1 | Poor |
| 4 | 0 " | 7.6 | " " | 17.0 " | 8.7 | " |

4–B. Condensed milk homogenized at 60° C. and pasteurized by heating to 61° C. and holding for 30 minutes

| | (a) Unhomogenized | | | (b) Homogenization pressure 1000 pounds | | |
|---|---|---|---|---|---|---|
| 0 | 20.0 Flat | 7.3 | Fat separation | 20.5 Flat | 7.7 | Good |
| 1 | 20.5 " | 7.4 | Objectionable. Flaky cream layer | 20.5 " | 8.0 | Some fat separation. Fair body |
| 2 | 20.0 " | 8.1 | " " | 20.5 " | 8.4 | " " |
| 3 | 19.0 Sl. oxidized | 8.6 | " " | 18.0 Oxidized | 8.9 | " " |
| 5 | 17.0 Oxidized | 9.1 | " " | 19.0 Sl. oxidized | 11.0 | " " |

5° C. (41° F.). The results reported above would seem to extend this range to – 17° C. On the other hand, Thurston, Brown, and Dustman (12) show that ". . . freezing and thawing under the conditions obtaining caused the reduction or elimination of the susceptibility of the milks to oxidized flavor development." It should be noted, however, that Thurston *et al.* used unhomogenized whole milk and they report further "whenever an excessive cream plug was observed the milk failed to develop oxidized flavor even with 3.9 parts per million of added copper, but when a less pronounced cream plug was observed some development of oxidized flavor usually was evident."

## CONCLUSIONS

1. In making unsweetened condensed milk to be stored at low temperatures, the enzyme lipase should be destroyed by heating prior to other processing steps so as to prevent development of a rancid flavor.

2. Unsterilized, condensed milk can be preserved satisfactorily in the frozen state longer than at 0° C. because bacteria are quiescent at the former temperatures but not at the latter temperature.

TABLE 4–A AND 4–B—(*Continued*)

*Effect of Homogenization Pressure on Properties of Condensed Milk Stored at –17° C.*

4–A. Condensed milk homogenized at 55° C. and pasteurized by heating to 61° C. and holding for 30 minutes

| Weeks in storage | (c) Homogenization pressure 2000 pounds | | | (d) Homogenization pressure 3000 pounds | | |
|---|---|---|---|---|---|---|
| | Flavor | Viscosity | Body | Flavor | Viscosity | Body |
| | | *Centipoises* | | | *Centipoises* | |
| 0 | 21.5 Flat | 6.7 | Good | 21.0 Flat | 7.7 | Good |
| 1 | 20.0 " | 6.8 | Better than (a), (b), or (d) | 20.0 " | 8.2 | Thin deposit on bottom of can |
| 2 | 20.0 " | 7.2 | Deposit on bottom of can | 20.5 " | 9.4 | Better than (a), (b), or (c) |
| 3 | 18.0 Bitter | 7.5 | Flaky condition. Fair body | 19.0 Sl. bitter | 22.2 | Sl. thickening. Poor body |
| 4 | 12.0 Oxidized | 10.4 | " " | 17.0 Oxidized | 27.7 | Flaky, cultured buttermilk body |

4–B. Condensed milk homogenized at 60° C. and pasteurized by heating to 61° C. and holding for 30 minutes

| | (c) Homogenization pressure 1800 to 2200 pounds | | | (d) Homogenization pressure 3000 to 4000 pounds | | |
|---|---|---|---|---|---|---|
| 0 | 21.0 Flat | 8.9 | Good | 21.0 Flat | 18.0 | Good |
| 1 | 21.0 " | 9.1 | " | 21.0 " | 18.5 | Good, viscous |
| 2 | 21.5 " | 10.2 | " | 21.5 " | 20.2 | " " |
| 3 | 21.0 " | 10.7 | " | 20.0 Off | 31.3 | Thickening in bottom portion |
| 5 | 20.0 Old | 17.6 | Creamy, cultured buttermilk body. Better than (d) | 20.0 Old | 74.6 | Creamy, cultured buttermilk body |

3. Other conditions being equal, condensed milk stored at –17° C. is more susceptible to the development of an oxidized, cappy, or tallowy flavor than is condensed milk maintained at –7° C.

4. In order to retard the development of an oxidized flavor in condensed milk that is to be stored for a period of several weeks, the milk should be exposed to as severe a heat treatment as possible without injury to the flavor and body. Under the conditions of these experiments best results were obtained when the condensed milk was heated at 76.5° C. for 8 minutes.

5. Homogenization prevents the formation of an undesirable layer of fat on the surface of condensed milk that has been frozen and aids in retarding changes which are indicated by an oxidized flavor. Pressures

of 2000 to about 3000 pounds per square inch are satisfactory. Higher pressures may be injurious to the body of the product.

6. Market milk may be preserved satisfactorily in the form of homogenized, frozen condensed milk for at least 2 weeks and in favorable circumstances for 4 weeks or longer.

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# MILK AND STARTERS FOR BRICK CHEESE

WILLARD LANGHUS,[1] PAUL R. ELLIKER AND WALTER V. PRICE
*University of Wisconsin, Madison, Wisconsin*

The irregularity of quality in commercial Brick cheese has been attributed partly to methods of manufacture and to a great extent to the type and quality of starters used. In 1936 a survey was made to study factory methods of making Brick cheese in Wisconsin. This study revealed that a wider variety of methods were used to encourage the development of acidity during the manufacturing process than was originally supposed. It was found that the ordinary *Streptococcus lactis* type of starter was commonly used in the making of cheese from pasteurized milk. Whey starter, however, was commonly used in raw-milk factories. Whey for starter was taken from the vat at the time of dipping, or a portion was saved after skimming. Sometimes the whey was simply set aside in a warm place to sour. Sometimes, especially in the summer, the whey was heated to approximately 150° F. then allowed to cool slowly and was put in a warm place to sour. At other factories the whey was heated to about 180° F. then cooled to 80° F. and inoculated with a small portion of whey saved from the previous day's starter.

## METHODS

It was the object of the study to determine the significant types of organisms and their approximate numbers in the starters and to study the methods of carrying the starters in several factories. At the same time observations were made on the types and approximate numbers of significant organisms in the milk supply. Methylene blue tests were made of milk delivered by each patron and in addition observations were made of the quality of the resulting cheese.

Twelve factories located in various parts of the Brick cheese area of Wisconsin were visited during August, 1937. Some of these factories were selected because they were making good cheese, others because they were having difficulty in making cheese of satisfactory quality.

One day was spent at each of the twelve factories in taking samples and making the necessary observations. A sample of starter, samples of milk from individual patrons, and a composite sample of milk representing the supply of the entire factory were obtained on that day. Laboratory work began within an hour or two after the cheese was made.

The starter samples were examined by dilution in litmus milk followed by incubation at 20°, 37° and 48° C.; by Burri slopes; and by microscopic

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examination. The composite samples of milk were treated in the same manner.

Samples of cheese made on the day when the milk and starter were studied were examined for quality and acidity seven or eight days after manufacture. These samples of cheese were obtained by making a second visit to each of the factories.

## QUALITY OF MILK

The distribution of patrons according to methylene blue tests of milk delivered at 6 of the 12 factories is shown in Table 1. One might question

TABLE 1

*Distribution of numbers of patrons by methylene blue test of milk delivered*

| Reduction time | Factory number | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 5 | 6 | 3 | 10* | 2* |
| *Hrs.* | | | | | | |
| 0.3 | 1 | 4 | ..... | ..... | ..... | ..... |
| 1 | 7 | 4 | 3 | ..... | ..... | ..... |
| 2 | 8 | 15 | ..... | 2 | ..... | ..... |
| 3 | 3 | 2 | ..... | ..... | ..... | ..... |
| 4 | 5 | 4 | 12 | 3 | ..... | ..... |
| 5 | 8 | 6 | 1 | 10 | 4 | 3 |
| 6 | ..... | ..... | 4 | ..... | 4 | 2 |
| 7 | ..... | ..... | ..... | ..... | ..... | 2 |
| Average time | 2.9 | 2.5 | 4.0 | 4.4 | 5.5 | 5.9 |

* Milk delivered twice daily.

the meaning of average reduction times which have been calculated for all factories. Average methylene blue reduction times of the patron's milk and estimates of the quality of the cheese are shown in Table 2. Observations in the Swiss and American cheese industries indicate that the chances of producing cheese of high quality are much better when the milk with a long reduction time is delivered to the factories. This relation undoubtedly exists for Brick cheese but is especially obscured in this study by the

TABLE 2

*Relation between average methylene blue test and quality of cheese*

| Factory no. | Reduction time | Quality of cheese | Factory no. | Reduction time | Quality of cheese |
|---|---|---|---|---|---|
| | *Hrs.* | | | *Hrs.* | |
| 8 | 1.7 | Good* | 7 | 4.4 | Fair |
| 5 | 2.5 | Poor | 3 | 4.4 | Good |
| 1 | 2.9 | Poor | 10† | 5.5 | Poor |
| 11 | 3.3 | Good | 9† | 5.9 | Fair |
| 4 | 3.4 | Fair | 2† | 5.9 | Good |
| 6 | 4.0 | Poor | 12† | 6.6 | Poor |

* Milk was pasteurized before manufacturing.
† Milk was delivered twice daily.

observations made at those factories where milk was delivered twice daily. Standards of quality for such factories, in terms of methylene blue test, must be different from the acceptable standard for other factories. The data of Table 2 simply show that factors other than mere numbers of organisms influence the quality of the resulting cheese.

Important types of organisms and their approximate numbers in milk supplies of poor, fair and good qualities, are shown in Table 3. Data on

TABLE 3

*Approximate numbers of important types of organisms in the milk supplies of three different factories*

| Types of organisms | Numbers of organisms detected and classified by litmus milk dilution tubes and microscopic examination | | |
|---|---|---|---|
| | Factory 1 Poor milk supply | Factory 7 Fair milk supply | Factory 2* Good milk supply |
| S. lactis | 100,000,000 | 10,000,000 | 1,000 |
| S. thermophilus | 1,000,000 | 100,000 | 10,000 |
| Lactobacillus | 100,000,000 | 10,000 | 100,000 |
| Yeast | 1,000,000 | none | none |
| Escherichia-Aerobacter | 10,000,000 | 100 | none |
| Average reduction time | 2.9 hours | 4.4 hours | 5.9 hours |

* Milk delivered twice daily.

the milk supply of factory 1 indicate growth of organisms probably induced by lack of cooling or by faulty conditions of production and handling. Observations at factory 7 indicate that the milk is fairly clean but probably lacks cooling. It is interesting to note the relatively small numbers of the *Streptococcus lactis* type of organisms in the milk of factory 2. This factory receives milk from the farmers twice each day and whey is returned to the farms in the milk cans. Since the milk is brought to the factory immediately after it is drawn from the cows there is little or no attempt made to cool it on the farms. It is not surprising, therefore, to find rather large numbers of the *Streptococcus thermophilus* and *Lactobacillus* types of organisms in these milk supplies.

Methylene blue tests of the factory milk supply and the type of information illustrated in Table 3 might be used to estimate the quality of the milk delivered to each factory. The factors considered in making such an estimate would be the number and types of organisms as indicated by methylene blue tests and dilutions in litmus milk and relative numbers of acid-producing and *Escherichia-Aerobactor* types. Application of this method of estimating the quality of the 12 milk supplies studied places in the "undesirable" or "poor" class some supplies which were satisfactory according to the methylene blue test alone. This estimate of quality seemed to be a little more closely related to the quality of the cheese than the methylene blue test alone. Despite this more complete information upon

milk quality which these two examinations afforded, still the prediction of cheese quality from milk quality was unreliable enough to indicate that other important factors were involved.

## QUALITY OF STARTERS

The visits to the twelve factories disclosed that nine of the twelve were using pasteurized whey for starter. These whey starters were generally characterized by a rather sweet odor although three of them had a flavor suggesting butyric acid or yeast. These pasteurized-whey starters were made by taking whey from the cheese vat and heating it in a shot gun can to a temperature of about 145° F. The container of hot whey was then set aside to cool to room temperature. Naturally temperature changes were fairly rapid so that the holding time at the effective pasteurizing temperature was sometimes relatively short. The types and numbers of organisms surviving such a heat treatment depend upon the types and numbers of organisms present in the whey before the heating process. Only one operator inoculated the whey after pasteurizing and he simply added a portion of starter saved from a previous batch. If the quality of the milk were very desirable and if acid-producing thermoduric organisms were always present in large numbers this method of carrying starter might be justified. In actual practice, however, it actually propagates the undesirable as well as the desirable types and eventually defective cheese results.

Temperatures of heating curd and whey in the cheese vats ranged between 108° and 118° F. Even the lowest temperature of 108° F. retards the activity of the *Streptococcus lactis* type of organisms but permits the growth of *Streptococcus thermophilus* and the *Lactobacillus* types. It would be expected, therefore, that large numbers of these thermophilic types of organisms should survive the cheese making process and be present in the whey at the time of pasteurizing. Relatively large numbers of such types, therefore, would also be expected to survive the heat treatment and cause acid development in the pasteurized-whey starter. Evidence of the survival of these thermoduric types of acid-producing organisms is presented in Table 4. The approximate numbers of the important types of organisms present in three typical starters are shown in this table. Undesirable as well as desirable types were found. Yeasts were present in four of the whey starters examined. Gas-forming organisms of the *Escherichia-Aerobacter* type were found in three of the nine starters made from whey. The presence of these undesirable types might be attributed to inefficient pasteurization or to contamination after the heat treatment. Although all nine of the factories making pasteurized-whey starters heated the milk in metal containers all of them but one transferred the whey to stone jars in which the whey was held in a warm place while it developed the desired acidity. Such utensils are practically impossible to keep bacteriologically clean and sterilized under ordinary factory conditions.

TABLE 4

*Approximate numbers of important types of organisms in three typical starters*

| Types of organisms | Number of organisms detected and classified by litmus milk dilution tubes and microscopic examination (000—omitted) | | |
|---|---|---|---|
| | Factory 5 Poor starter | Factory 4 Fair starter | Factory 3 Good starter |
| S. lactis | 1,000,000,000 | 1 | 100,000 |
| S. thermophilus | 10,000 | 1,000,000 | 1,000,000 |
| Lactobacillus | 10,000 | 1,000,000 | none |
| Yeast | 10 | 10 | none |
| Escherichia-Aerobacter | 1,000 | none | none |

Three factories did not use pasteurized-whey starters. One was using *Streptococcus lactis* in milk, and the other two were using *Lactobacillus bulgaricus* in milk. These starters were made by heating milk in a steam or hot water bath for a period of from one to two hours. The milk was cooled, transferred to a stone jar and inoculated with a mother culture. This mother culture was prepared by simply holding a portion of the bulk culture in a fruit jar until the heated milk was ready for inoculation later in the day. Yeast contaminated two of the three milk starters examined.

Table 5 illustrates the methods of handling them and some of the char-

TABLE 5

*Propagation and characteristics of typical starters*

| Factory | 5 | 4 | 3 |
|---|---|---|---|
| Starter type | Heated whey | Heated whey | Heated whey |
| Inoculation | None | None | Whey starter |
| Incubation | Boiler room | Boiler room | Make room |
| Temperature (Approx.) | 85° F. | 85° F. | 75° F. |
| Acidity | 0.41 per cent | 0.36 per cent | 0.34 per cent |
| Odor and flavor | Tainted | OK. | OK. |

These factories:—(1) Heated whey in shot gun cans to 145° F.; (2) allowed whey to cool in air; (3) placed whey in stone jars to acidify.

acteristics of typical starters observed in these factories. The most useful of all of the observations is probably the record of acidity obtained at the time each starter was used in the manufacture of cheese. This acidity is regarded as a general indication of the activity and vitality of the starter. By taking into consideration, therefore, the dominant types of organisms discovered in the starter and the acidity attained at the time the starters are ready for use as well as the characteristic flavor one might estimate the vitality or activity and consequently the usefulness of the starters. Whey starter in which *Streptococcus thermophilus* is the dominant type should attain an acidity of approximately 0.45 per cent, following the usual overnight holding period under factory conditions. Whey starters with the *Lactobacillus* types of organisms predominating should attain an acidity of approximately 0.8 per cent. Starters made with milk and inoculated with *Streptococcus lactis* should have an acidity at the time of using of approxi-

mately 0.75 per cent, while an active *Lactobacillus* starter should have an acidity of 1 per cent.

Estimates of the quality of the cheese obtained in these factories were made in terms of three general levels, namely: "poor," "fair" and "good." In Table 6 these general estimates of cheese quality are presented with the

TABLE 6

*Quality of milk, starter and cheese*

| Factory no. | Quality of milk | Quality of starter | Quality of cheese | |
|---|---|---|---|---|
| | | | Estimate | Comments |
| 3 | Poor | Good | Good | Long, smooth |
| 8 | Poor† | Fair | Good | Long |
| 11 | Fair | Fair | Good | Firm, close, sweet‡ |
| 2* | Good | Fair | Good | Close, sweet |
| 7 | Fair | Good | Fair | Sweet, sl. open, few pin holes |
| 9* | Fair | Fair | Fair | Long, few pin holes, sl. unclean |
| 4 | Poor | Fair | Fair | Sweet, pin holes, sl. unclean |
| 10* | Fair | Fair | Poor | Sl. short, pin holes, curdy, unclean |
| 12* | Poor | Fair | Poor | Sl. short, pin holes, sl. unclean |
| 1 | Poor | Fair | Poor | Open, pin holes, unclean |
| 5 | Poor | Poor | Poor | Open, pin holes, unclean |
| 6 | Poor | Poor | Poor | Open, pin holes, unclean |

* Milk was delivered twice daily.

† Milk was pasteurized before making into cheese.

‡ Some lots of cheese in this factory were developing openness and splitting at 2 weeks of age. The defect was not found in this cheese at 7 days of age. The starter, however, contained spore-forming organisms which have been found to be associated with this defect in this laboratory.

corresponding estimates of the quality of the milk and the quality of the starters which were used in making the cheese. Apparently when starter of good quality is used with milk of inferior quality the chances of producing satisfactory cheese seem to be greatly improved. In no instance was a poor starter associated with the manufacture of a good cheese. The quality of the cheese in general was no better than the quality of the starter used in its manufacture.

Our observations seem to indicate, even though the relationship between the quality of the milk and the quality of the resulting cheese is not infallible, that care used in the selection of milk for Brick cheese should result in better cheese. The results of this study indicate definitely that an improved method of propagating starters in cheese factories is needed. The fact that the quality of the starter may tend to limit the quality of the cheese which can be produced from any given milk supply would certainly seem to justify the extra effort and attention necessary to produce starters of the highest quality for the manufacture of Brick cheese.

## CONCLUSION

Starter used in the commercial manufacture of Brick cheese could be improved by better methods of propagation. The cheese is usually no better than the quality of the starter used in its manufacture.

# ARTIFICIAL INSEMINATION IN DAIRY CATTLE[1]

C. L. COLE
*University of Minnesota, Grand Rapids, Minnesota*
AND
L. M. WINTERS
*University Farm, St. Paul, Minnesota*

The first work in artificial insemination at Minnesota was done with sheep. Technics for semen collection and for the insemination of the ewes were developed. Solutions and methods for the preservation of sperm were also perfected and ewes have been successfully inseminated with sperm six days of age although they have been kept motile for much longer periods of time. We have successfully transported semen back and forth between the Sub-Station at Grand Rapids and University Farm, St. Paul, Minnesota. During the past fall we also successfully shipped semen from Grand Rapids, to the George McKerrow and Sons Farm at Pewaukee, Wisconsin.

During 1935 and 1936, considerable attention has been given to developing technics for the insemination of cattle at the University of Minnesota. During the Spring of 1937 it was thought that enough preliminary work had been completed to give it a field trial. In June of 1937, a small breeding route was organized around the Station at Grand Rapids.

## METHODS OF COLLECTING SPERM

Three methods have been used for semen collection:

1. Allowing the bull to serve a cow out of heat. This method has the particular advantage of being very easy and requiring no special equipment except a long glass pipette with a rubber bulb attached.

There are some disadvantages, however: (1) It is often difficult to train an aged bull to serve a cow out of heat. (2) Considerable semen is lost in the folds of the vagina. (3) Semen samples are often contaminated with vaginal discharges. (4) Great care must be used in selecting a host female that is entirely free from any disease or infection that may contaminate the semen.

2. The second method and one that has been used very extensively at Grand Rapids is to massage the ampullae. This method of collection offers some obstacles in that it is sometimes difficult to teach old bulls to react and the technics of successful massage require careful study and considerable practice so that the semen will not be contaminated with urine or carry undue quantities of seminal fluids. The particular advantages are: (1) The semen is obtained easily and quickly after the operator has gained

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experience and the animal has become trained. (2) There is no danger of female contamination: no special equipment is required. (3) What is of most importance, proved bulls that for some reason or other are unable to mount a cow may be kept in service.

3. The third method being used is collection with an artificial vagina. This method was first developed in Russia and has been used with considerable success at Cambridge and other places.

The bull is allowed to mount a blanketed cow and the penis directed into the mouth of the artificial vagina where a normal ejaculation occurs. This method requires some special equipment and considerable care in the use of that equipment. It does, however, provide a way for the complete recovery of a normal ejaculate. Its use undoubtedly results in the best samples. They are uncontaminated by the female; furthermore, urine and excessive quantities of seminal fluids from the male are avoided. The seminal vesicles are often stimulated excessively by the massage method.

It would appear, however, that both of the latter methods will prove very useful to the successful collection of samples on a large scale.

## METHOD OF INSEMINATION

The inseminating pipette which we have used is a glass tube about seventeen inches long, $\frac{1}{4}$ inch outside diameter with a $\frac{1}{16}$ inch bore. This is welded at right angles to a tube about five inches long with a $\frac{3}{16}$ inch bore. It is advantageous to have a pocket blown in this piece to serve as a receptacle for the semen. A pyrex glass speculum $1\frac{1}{4}$ inches in diameter and fourteen to sixteen inches long and a flashlight complete the equipment. It is an advantage to the operator to have the speculum made with a light fastened in the end. All the inseminating equipment which we have developed is glass. This provides for easy cleaning and sterilization and low replacement cost. The speculum is used to locate the cervix and the semen is placed directly into the opening; one-half cc. of semen is sufficient to successfully inseminate a cow providing it is placed in the cervix.

When several cows are to be inseminated from one sample or if it is to be held for some time, the semen should be allowed to stand at room temperature for about twenty minutes and then diluted with an equal quantity of diluting solution. The semen is then put into a cooling chamber at 40° to 45° F. until ready for use. After removal from the cooling chamber, it is again allowed to stand at room temperature for 15–20 minutes and then held until it is approximately body temperature.

The normal ejaculate from a bull ranges from 4 to 10 or more cc. Doubling this amount with diluting fluid and using one-half cc. per female will allow inseminating from 16 to 40 females from one service.

The large majority of the cows in our breeding route have been inseminated at the end of oestrum or shortly after. Available data indicate that

the bovine ovulates approximately twenty-four hours after passing off heat so that this would seem to be the optimum time for insemination.

### DATA ON COWS

A total of 121 cows have been inseminated. Of these, 105 are definitely pregnant, 12 have been inseminated too recently to be certain of pregnancy, and four have been discarded as non-breeders. The miscellanous group as shown in Table 1 has the highest breeding efficiency with a 1:1.32 average.

TABLE 1

*Insemination record*

| | No. of cows inseminated | No. of inseminations | No. of cows pregnant | No. of cows inseminated too recently to be sure of pregnancy | No. of cows failing to conceive | Breeding ratio for cows definitely pregnant |
|---|---|---|---|---|---|---|
| Misc. Group[1] | 39 | 53 | 34 | 5 | .. | 1:1.32 |
| Herd No. I[2] | 39 | 55 | 37 | 1 | 1 | 1:1.43 |
| Herd No. II[3] | 25 | 51 | 21 | 4 | .. | 1:2.23 |
| Herd No. III[4] | 18 | 48 | 13 | 2 | 3 | 1:3.15 |
| Totals | 121 | 207 | 105 | 12 | 4 | 1:1.77[5] |

[1] Cows owned in several small herds.

[2] Previous five-year average was 1:2.

[3] This herd has been in the process of Bang's disease elimination during the entire period of insemination.

[4] This herd has had considerable breeding trouble for several years and artificial insemination was resorted to as an emergency measure.

[5] The four cows that were discarded as non-breeders were inseminated 13 times and are figured in the ratio.

Herd No. I with an efficiency of 1:1.43 shows a decided increase over the previous five year average which was 1:2. Herd No. II has been having a great deal of difficulty with Bang's disease and at least one-third of the cows inseminated were reactors. Herd No. III has had considerable breeding trouble for many years and artificial insemination was resorted to as an emergency measure. Herds II and III do not represent a true picture of the possibilities of artificial insemination but were used in this trial because numbers were needed.

Sixty-four of these cows settled on their first insemination, 27 on their second, and only 13 required more than two inseminations. Eleven of the 13 requiring more than two inseminations were in Herds II and III and three of the four non-breeders were in Herd III.

Artificial insemination is not an emergency measure for getting cows in calf and should not be used for that purpose only. Its successful use will depend on proper supervision and control when it is used in regularly organized breeding groups as a regular procedure. These breeding groups

should be under direct supervision of the university of the state in which the work is being done. Specific control measures should provide for the proper sanitation with regard to operators, instruments, and animals. Sanitation cannot be stressed too much if the program is to operate successfully and be a disease control measure. We do not believe it necessary to have a veterinarian specified as the only person qualified to be an operator. Any reliable person drilled in the essentials of sanitation and possessing a knowledge of the reproductive tract should be qualified.

These control measures should also provide for the inspection and certification of any bull to be used in the breeding group.

The successful insemination of a thousand or more cows per year to one sire is not at all beyond definite realization.

Artificial insemination offers to the cattle breeders a tool that gives them an opportunity for the most rapid improvement that has ever been known in cattle breeding. When it is organized as a part of a regular improvement program including butterfat testing, it offers a sure and quick method for proving young sires without special loss or gain to any one breeder and an opportunity to greatly prolong and increase the use of sires that have already proved their merit.

# STABILITY OF CAROTENE IN GREEN GRASSES AND ALFALFA STORED AT FIVE DEGREES FAHRENHEIT[1]

DONALD W. BOLIN

*Idaho Agricultural Experiment Station, Moscow, Idaho*

Samples of alfalfa and different varieties of grasses were stored fresh at 5° F. with the assumption that very little or no loss of carotene would occur at this temperature. Several samples were analyzed before putting into cold storage and at the end of 10 months' storage the samples were again analyzed for carotene. There was a definite loss of carotene in the alfalfa samples with little or no loss of carotene in the grass samples. No data could be found in the literature on the stability of carotene in different green forages stored at low temperatures. Data are presented to show that the stability of carotene varies with the type of green forage when stored over a period of time at 5° F.

## EXPERIMENTAL PROCEDURE

Alfalfa (*Medicago sativa* L.) and four different varieties of grasses, namely, Kentucky blue (*Poa pratensis* L.), orchard grass (*Dactylis glomerata* L.), meadow fescue (*Festuca elatior* L.) and brome (*Bromus inermis*), were cut green at two weeks' growth at weekly intervals for four weeks during the month of July, immediately packed with dry ice (solid carbon dioxide), sent to the laboratory, and ground green with the aid of dry ice in a Wiley mill. The samples, while still frozen, were thoroughly mixed and a portion of the samples taken for immediate carotene analysis. The other portion of the samples was packed in Mason jars, the lids of which were screwed on loosely to allow the excess $CO_2$ to escape. The jars were then put back into the original shipping carton to keep out light and immediately put in cold storage at 5° F. while the forage was still frozen. The carotene was determined according to the method described by Guilbert (1) with slight modifications. All calculations are based upon the oven dry basis and the carotene content expressed as milligrams of carotene per 100 grams of forage.

## RESULTS AND DISCUSSION

The results of the analyses of the carotene in the forage before and after storage are shown in Table 1. It is to be noted that the loss of carotene in the grasses is negligible. Any difference in the amount of carotene present before and after storage may be due entirely to experimental error in the

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[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 173.

TABLE 1

*The carotene content (milligrams per 100 grams of forage on oven dry basis) in forage before and after 10 months' storage at 5 degrees Fahrenheit*

| | Date collected | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July 8, 1936 | | | July 15, 1936 | | | July 23, 1936 | | | July 29, 1936 | | |
| | Before storage | After storage | Difference | Before storage | After storage | Difference | Before storage | After storage | Difference | Before storage | After storage | Difference |
| Kentucky blue | 19.5 | 19.5 | 0 | 27.0 | 28.6 | + 0.4 | 28.5 | 29.3 | + 0.8 | 43.0 | 36.3 | −6.7 |
| Orchard grass | 34.4 | 33.8 | − 0.6 | 41.4 | 41.0 | − 0.4 | 47.5 | 44.2 | − 3.3 | 35.3 | 38.5 | +3.3 |
| Meadow fescue | 28.6 | 29.5 | + 0.9 | 54.7 | 48.1 | − 6.6 | 50.0 | 45.1 | − 4.9 | 49.3 | 42.1 | −7.2 |
| Brome | 36.2 | 40.5 | + 3.7 | 48.6 | 50.5 | + 1.9 | 57.5 | 50.2 | − 7.3 | 43.3 | 39.2 | −4.1 |
| Alfalfa | 21.7 | 8.01 | −13.7 | 28.3 | 7.51 | −20.8 | 25.0 | 8.0 | −17.0 | 15.1 | 10.0 | −5.1 |

determination of the carotene. The alfalfa, however, lost, on the average, 62.7 per cent of its initial carotene.

Samples of alfalfa stored under the same conditions the following year showed an average loss of 70 per cent.

These results indicate that a carotene-destroying factor, active at 5° F., is present in the alfalfa and not in the grasses. Since all these samples of forages were stored under the same conditions, the possibility of either light or oxygen being the direct cause for the loss of carotene in alfalfa is apparently eliminated, as one would expect a similar loss of carotene in the grasses. Several samples of dehydrated alfalfa, stored under the same conditions, were analyzed for carotene and showed a loss of only one per cent for the storage period. This difference in the stability of carotene in dehydrated and green alfalfa would indicate that the destructive agent present in green alfalfa is an enzyme.

The results of this experiment indicate that the storage of certain forages at low temperatures does not prevent the loss of carotene, whereas, the storage of other forages studied under similar conditions will prevent this loss. The publication of these results, therefore, should be a warning to other investigators of the danger in storing forages at low temperatures for the preservation of carotene without giving due consideration to the type and condition of forage stored.

## SUMMARY

Carotene analyses of green alfalfa and green grasses before and after storage at 5° F. for 10 months show:

1. Alfalfa loses a large percentage of its carotene content.
2. Kentucky blue, orchard grass, meadow fescue and brome lose little or no carotene.

## REFERENCE

(1) GUILBERT, H. R. Determination of carotene as a means of estimating the vitamin A value of forage. Jour. Ind. & Eng. Chem., Anal. Ed. 6: 452. 1934.

# THE VITAMIN A CONTENT OF DRIED CITRUS PULP

M. C. FUTCH,* L. L. RUSOFF AND R. B. BECKER
*Florida Agricultural Experiment Station, Gainesville, Florida*

## INTRODUCTION

The assay of dried citrus pulp for vitamin A content has been one phase of the investigation of the nutritive properties of citrus by-products at the Florida Agricultural Experiment Station.

Work by Fraps and Triechler (1) indicated that dried orange peel and pulp contained four to six units of vitamin A per gram, and dried grapefruit peel and pulp less than five units per gram, however, these investigators used a limited number of animals and these had high mortality during the assay. Neal (2) observed avitaminosis A in a yearling steer fed a ration of citrus pulp and cottonseed meal (4:1).

## EXPERIMENTAL

The procedure outlined in the U.S.P. XI (3) was used in this study except for necessary modification for this type of test material. Normal piebald rats from the stock colony of the Florida Agricultural Experiment Station were used.

The citrus pulp, largely from grapefruit canneries, was dried in a parallel current, direct-heat, stationary drier, agitation of the product being accomplished by rotary paddle wheels. The pulp was ground in a Wiley mill through a one mm. sieve.

On the basis of preliminary assay trials, the final assay groups containing seven rats per group were assigned daily supplement levels of pulp as follows: Group I, 1.0 gram; Group II, 1.25 grams; and Group III, 2.0 grams. The control group was not fed any supplement. The reason for not carrying the assay on higher levels was due to the inability of the animals to consume more than two grams of the supplement daily.

## RESULTS AND DISCUSSION

The gain or loss in weight for all the groups during the assay period are presented in Figure 1.

Of all the rats which received the citrus pulp, only five rats on 2.0 grams of pulp daily finished the assay period, making an average gain of 5.0 grams for the period which indicates the presence of some vitamin A in the pulp.

Received for publication July 28, 1938.

* The data upon which this paper is based were taken from a thesis presented by M. C. Futch, in partial fulfillment of the requirements for the degree of M.S., University of Florida.

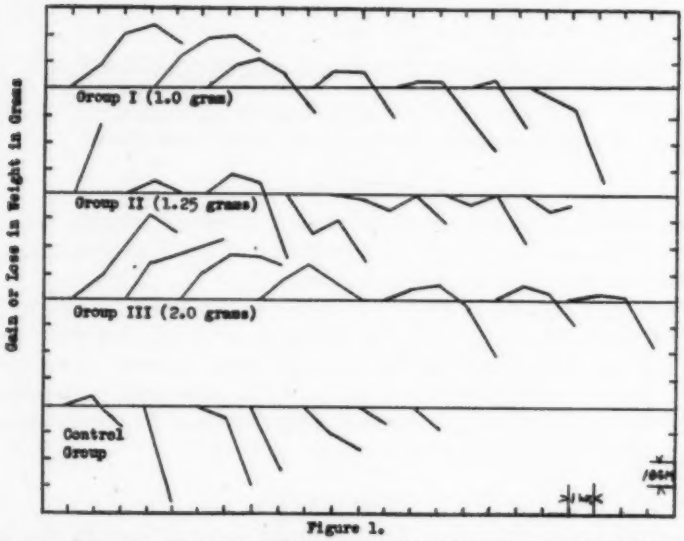


FIG. 1. Gain or loss in body weight of rats fed different amounts of citrus pulp supplemented to U.S.P. XI vitamin A test ration.

It is evident from the curves that the dried citrus pulp contained less than 0.5 unit of vitamin A (U.S.P. XI) per gram. This low content may be attributed to three causes; low content of vitamin A in the original material, partial destruction of the vitamin in the drying process, or destruction during the period of storage.

## SUMMARY

The dried citrus pulp used in this study assayed less than 0.5 U.S.P. XI units per gram of the product.

## REFERENCES

(1) FRAPS, G. S., AND TRIECHLER, R. Vitamin content of foods and feeds. Texas Agr. Exp. Sta. Bul. 477. 1933.

(2) NEAL, W. M. Unpublished work.

(3) PHARMACOPOEIA OF THE UNITED STATES OF AMERICA. First supplement, eleventh decennial revision, pages 75–82. 1937. Prepared by the committee of revision and published by the board of trustees. Agent, Mack Printing Company, Easton, Pa.

# American Dairy Science Association Announcements

THE STATE COLLEGE OF WASHINGTON AND THE UNIVERSITY OF IDAHO INVITES YOU TO ATTEND THE THIRTY-FOURTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

*To the Officers and Members of the*
*American Dairy Science Association:*

It is an honor, as well as a pleasure, for us to know that the American Dairy Science Association has decided to hold its next annual meeting in Pullman, Washington and Moscow, Idaho, June 26–30, 1939. In behalf of the State College of Washington and the University of Idaho we extend a hearty welcome to your Association and hope that a large number of you will avail yourselves of this splendid opportunity of visiting the fine institutions, farms, factories, and scenic wonders of our great Pacific Northwest. We feel certain that you will not only enjoy the traditional western hospitality of our people, but that you will be impressed with the great potentialities of this region with its tremendous natural resources, which are just now beginning to be developed on a magnificent scale. The Grand Coulee Dam, largest structure of its kind in the world, will interest you as will our forests, lakes, streams, mountains and coast.

Dairying is an important industry in this area and our people will be delighted to meet personally the men and women from the length and breadth of this country and others who are the leaders in the scientific advancement of this great industry. We shall spare no effort to make you, your families, and friends comfortable while here. May the 1939 meetings of the American Dairy Science Association surpass all others in attendance, in accomplishments and in personal enjoyment for all of you.

E. O. Holland, *President*
State College of Washington
Pullman, Washington

H. C. Dale, *President*
University of Idaho
Moscow, Idaho

THIRTY-FOURTH ANNUAL MEETING, STATE COLLEGE OF WASHINGTON, PULLMAN, WASHINGTON, AND UNIVERSITY OF IDAHO, MOSCOW, IDAHO, JUNE *27–30, 1939*

*Monday—June 26*

1:30 P. M.—General Registration and Room Assignment.

*Tuesday—June 27*

9:30 A. M.—Dairy Products Judging Conference for Coaches and Instructors.

1:30 P. M.–2:30 P. M.—Opening Session.

2:30 P. M.–4:00 P. M.—General Session (Education).
4:00 P. M.—Meeting of the Board of Directors, and Committee Meetings.
8:00 P. M.—Social Hour.

*Wednesday—June 28*

8:30 A. M.–9:30 A. M.—Committee Meetings.
Inspection of Extension Exhibits.
9:30 A. M.–12 NOON—Sectional Meetings.
1:30 P. M.–4:00 P. M.—Sectional Meetings.
4:00 P. M.—Committee Meetings.
8:00 P. M.—Reception.

*Thursday—June 29*

8:30 A. M.–9:30 A. M.—Committee Meetings.
9:30 A. M.–12 NOON—Sectional Meetings.
1:30 P. M.–4:00 P. M.—Sectional Meetings.
4:00 P. M.—Committee Meetings.
6:30 P. M.—Annual Banquet.

*Friday—June 30*

8:30 A. M.–12 NOON—General Session and General Business Meeting.

### CALL FOR TITLES AND ABSTRACTS OF PAPERS

Members of the Association are invited to submit titles and abstracts of papers dealing with original investigations. Those interested in presenting papers should refer to page 789 of the December, 1937, number of the Journal for pertinent information. Time allotted for each paper is limited to 10 minutes. Titles and abstracts of papers must be in the hands of the chairman of the program committee by April 15.

All communications relative to the program should be addressed to Vice-Dean E. V. Ellington, Department of Dairy Husbandry, State College of Washington, Pullman, Washington.

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# ABSTRACTS OF LITERATURE

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# ABSTRACTS OF LITERATURE

**30. Cattle Fodder and Human Nutrition.** ARTURI I. VIRTANEN. Cambridge University Press; New York, The MacMillan Co., 108 pages, illus. 1938. $2.25.

This book comprises four lectures the author delivered at the Universities of London, Reading, and Cambridge during 1937. The lectures were: I. The Mechanism of the Biological N-Fixation, II. The Symbiosis of the Leguminous Plants with the Legume Bacteria, III. The Production of Vitamins in Agriculture, with Special Reference to Human Nutrition, and IV. The A. I. V. Method for the Preservation of Fresh Fodder and Its Importance in Agriculture. The appended General Survey summarizes and correlates the material of the four lectures which might otherwise have appeared somewhat unrelated. Included in the Survey is a diagram of the author's conception of the cycle of nitrogen changes as related specifically to the fixation and excretion of nitrogen by plants and the utilization and excretion of nitrogen by animals.

The author, Professor at the Biochemical Institute at Helsinki, Finland, is eminently qualified to discuss the material presented in this small volume. He has initiated and directed many investigations which have originated or stimulated the development of new techniques for the study of nitrogen fixation problems. Of equal importance have been his studies resulting in the development and practical application of new methods. His statements and conclusions regarding the A. I. V. method of ensiling are scientifically correct and have repeatedly been verified by other scientists working under like conditions. Climatic conditions in this country permit the ensiling of the various crops at a higher dry matter content thereby preventing any loss of juice from the silo when proper precautions have been taken. For this reason the acid treatment of silage crops is of less significance in this country than in western Europe. However, in our experience the A. I. V. process is probably entitled to the highest rank among the various preservative treatments for forage crops with respect to the preservation of the carotene content of the green crops.

In his own country he has been unusually successfull in making extensive practical use of his investigations and he has inspired much recent work in other countries.

Although the author includes a general review of the recent literature in the fields discussed, undoubtedly his readers will welcome particularly his evident attempt to make available in the English language the methods and conclusions of his own investigations, thus far too little known. With slight modifications, the General Survey might well have formed an Intro-

duction to the book. The material has been carefully organized and well illustrated. A more appropriately chosen title would undoubtedly have resulted in the book receiving a more wide-spread and well-deserved reception. A.E.P. and H.W.B.

31. **Casein and Its Uses.** HANS HADERT. (Translation of second German edition by Dr. Henry Goldsmith.) Chemical Publishing Company of New York, 1938. Price $3.00.

In this book of slightly more than 100 pages the author has assembled a surprisingly large amount of useful and practical material on the manufacture and uses of casein. The book is written from the viewpoint of a technical expert in this field, and as its translator states in his preface it should be a valuable handbook for technicians in the casein industry and to those interested in the industrial uses of this product.

The first chapter covers briefly the various processes of manufacture of both acid precipitated and rennet type caseins. Flow sheets are given and typical plant operations are described. Factors affecting the quality of the product are discussed. Since the author is acquainted chiefly with German plants most of the machinery mentioned is obviously of German make, but the operations described are, of course, similar to those used with American machinery. German standards for casein and methods of testing casein are given in detail.

Some thirty pages are included in Chapter II—"Casein Adhesives." The grades or casein to be used, factors affecting viscosity, action of the numerous possible dissolving agents, waterproofing ingredients, etc., are discussed. This chapter assembles a large number of facts of practical value from a wide survey of the field, and gives numerous references to the literature as recent as 1936. Some attention is given to adhesives made by mixing other animal and vegetable proteins with casein, such as blood albumin, soy bean protein, ivory nut flour, castor bean protein, etc. The patent literature of this field in Germany and other countries seems to have been extensively covered by the author in his survey. Numerous practical recipes and mixes are given in detail for the preparation of adhesives for specific purposes.

Casein paints and color binders are the subjects of the third chapter. It is given over to a discussion of the various ingredients which may be used in the manufacture of these products. Again a large number of recipes is given, and a wide survey of the latest improvements and newest ingredients suggested in this field is condensed into a brief space, thus making available to the reader the most recent developments.

The concluding chapter—"Other Applications of Casein"—touches briefly on the use of casein in paper making, in plastics, textiles, medicines,

food products, etc., mentioning also recent work on the textile fiber, "Lanital." Most of this chapter, however, is given over to some 30–40 miscellaneous formulas using casein in various mixtures to be used for various purposes from paper sizing to cellular concrete mix.

As a whole the book accomplishes what the author intended in furnishing a handy and compact technical reference summarizing the industrial phases of the manufacture and use of casein. However, most of the emphasis is placed upon adhesives and paints as the major casein using industries, and persons interested in casein plastics, or other casein products will find less use for the book in their particular fields.

This edition of the book may be criticized somewhat on two points. The translator seems to have clung a little too closely to the literal translation at many points, which results in some rather unusual English equivalents; indeed, in some cases, in actual misleading statements. The second point is that the whole book gives one the impression of having been printed without the benefit of careful proof-reading. These are minor matters, however, and the book should prove of general interest to all who are interested in the casein industry, and particularly to manufacturers and technicians in the adhesive and paint phases of it. E.F.A.

**32. Utilization of Fats.** A Theoretical and Technical Treatise on the Composition, Analysis and Applications of Fats. H. K. DEAN, with a foreword by T. P. HILDITCH. 292 pages, Chemical Publishing Company, 148–H Lafayette Street, New York City. 1938. Price $6.00.

The industrial applications of fatty materials and their derivatives are described on the basis of a physico-chemical approach. The primary consideration is to present a clearer picture of the relation of structure of fats to their utilization, achieved largely by the replacement of the usual tables of iodine, saponification and other average values and the classification based upon them by tables showing the composition of fats in terms of fatty acids, and where possible glycerides. Titles of the seven chapters are: Constituents and Properties of Fats; Analytical Methods; Classification, Composition and Analysis of Fats; Artificial Fats; Extraction and Refining of Fats; Edible Fats; Paint and Varnish Oils; Soaps and Fatty Acids; Miscellaneous Applications, including Pharmaceutical Fats, Cosmetics, Lubricants, Leather and Sulfonated Oils. The inclusion of the fat soluble vitamins and the rancidity problem extend the interest of the book to a wider circle. J.F.L.

## FOOD VALUE OF DAIRY PRODUCTS

**33. Is Cobalt of Any Significance in the Treatment of Milk Anemia with Iron and Copper?** E. J. UNDERWOOD AND C. A. ELVEHJEM, De-

partment of Biochemistry, University of Wisconsin, Madison. J. Biol. Chem. *124*, 419, 1938.

Cobalt in exceedingly minute quantities is active in animal metabolism, and in comparatively large doses induces polycythemia.

It was concluded from the study that iron, copper and manganese are the only minerals which must be added to milk under the conditions of the experiment for the normal growth and well being of the rat, and that small amounts of cobalt, which contaminate almost all iron salts, play no significant part in the treatment of milk anemia in the animal. K.G.W.

**34. Soybean Products as Competitors of Dairy Products.** SYBIL WOODRUFF, Univ. of Ill. Nat. Butter and Cheese Jr. Vol. *29*, No. 20, p. 30, Oct. 25, 1938.

Soybean contains 40% protein and 20% oil, approximately. Crushing 100 pounds of soybeans yields about 12.5 pounds of oil. The uses of this oil as a food over a period of five years is indicated in this table.

*Factory Consumption of Soybean Oil for Food and Industrial Uses Over a Period of Five Years*

| Year | Lard compounds and vegetable shortenings | Margarines | Other edible products | Total | Total industrial uses |
|---|---|---|---|---|---|
| | Thousand pounds | Thousand pounds | Thousand pounds | Thousand pounds | Thousand pounds |
| 1932 | 4,889 | 3 | 180 | 5,072 | 19,039 |
| 1933 | 489 | 7 | 460 | 956 | 21,135 |
| 1934 | 2,735 | 24 | 509 | 3,268 | 16,816 |
| 1935 | 52,452 | 1,740 | 9,421 | 63,613 | 22,085 |
| 1936 | 113,897 | 14,262 | 21,598 | 149,757 | 25,847 |

Refined oil may show flavor changes, sometimes called fishy, that restrict its saleability as food. Six pounds of soybean press cake are produced with every pound of oil. It is used chiefly for livestock, but some is ground into flour or coarse grits for human consumption. Several varieties of soybeans are used as vegetable either green or dried. Soybean milk resembles cow's milk in appearance and contains proteins, carbohydrates and minerals which are extracted from ground, raw soybeans with water. Although the flavor is strange the milk may be used for cooking purposes and has been used by people allergic to cow's milk. Although the food uses of soybeans may be extensive according to present knowledge of its nutritive value soybean protein can be advocated only as a supplement to animal proteins.

W.V.P.

**35. Foods, Fads, and Fallacies.** TATE MILLER, M.D., Dallas, Texas. Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 73, 1937.

With the exception of the dietary treatment of diabetes and the diet of babies 90% of what the general public thinks about diet is pure bunk. The advertising claims for 400 different foods causes the consumer to wonder what he should eat. One cannot be guided by advertisements as to food or health directions. This is an interesting and readable discussion of food, fads, and fallacies. E.F.G.

## ICE CREAM

**36. Vanilla "As They Like It."** T. B. HARRISON, Univ. of Tennessee. Ice Cream Trade J., pp. 34, 10, 25, October, 1938.

Consumer preference tests were made at the University of Tennessee on ice cream submitted at different times by Tennessee manufacturers. Fat, total solids, acidity tests were made and flavor, body and texture defects noted. The testers were requested to place the samples in order of their preference. Five such tests were made with 57 to 73 people judging each set of samples.

Flavor proved to be the most important factor in the consumer choice of sample. Outstanding flavor defects were usually identified by the consumer and resulted in a low rating. In some instances pronounced pure vanilla flavor seemed to be preferred to a flat or unnatural vanilla flavor. Samples with too deep color and high acidities were placed down. The fat content of the samples ranged from 9 to 18 per cent, and had some effect on consumer preference but not as marked as flavor and body and texture defects. The type preferred by the majority of consumer judges was described as that having a true vanilla flavor, free from off flavors of dairy products, smooth in texture, low in acidity, and a pale yellow color.

W.H.M.

**37. Malted Milks.** MAL PARKS. Ice Cream Trade J., pp. 34, 11, 20, Nov., 1938.

Salesmen who suggest to their dealers that they lower the price of malted milks will usually find that the dealer cuts down on the amount of ice cream used in each serving and reduces the quantity and quality of other ingredients used.

The making of a good malted milk requires that all ingredients must be fresh and pure, the proper amount of each ingredient should be used, the milk must be very cold, and the drink must be allowed to mix for a suitable length of time.

Directions given for a good malted milk are as follows: 1. Put 1.5 oz. of syrup into a clean, shining mixing cup. 2. Add 6 oz. of very cold milk. 3. Add 2 No. 20 scoops of ice cream and ½ oz. malted powder. 4. Mix at least 1 minute. 5. Serve in a clean sparkling glass, dressed with whipped cream, placed on saucer, with a doily and malted milk cookies. The cost price on this malted milk is estimated to be 10¼ cents. W.H.M.

**38. Specialties.** J. H. FRANDSEN, Mass. State College, Amherst. Ice Cream Trade J., pp. 34, 11, 29, Nov., 1938.

The author suggests many ways of increasing ice cream sales. A list of special forms of ice cream which can be used for many occasions throughout the year is presented. W.H.M.

**39. The Importance of Quality Syrups.** MALCOLM PARKS. Ice Cream Trade J., pp. 34, 10, 70, October, 1938.

It has been found practical for the ice cream manufacturer to supply the dealer with paper and glass containers for sundae topping to be sold with ice cream for home use. This plan has stimulated the bulk package business in many stores. W.H.M.

## MILK

**40. A Survey of the Progress of Pasteurization.** JAMES A. TOBEY, The Borden Company, New York City. Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 90, 1937.

The history of pasteurization of milk is reviewed and the present extent of the practices is evidenced by the fact that in 1931 there were 99 cities in which all milk except certified was pasteurized. It is stated that pasteurization offers a guarantee of the safety of the milk supply which will increase consumption. E.F.G.

**41. The Sales Possibilities of Homogenized Milk.** W. A. MACDONALD, Pres., MacDonald Dairy Co., Flint, Mich., Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 99, 1937.

After analyzing the milk sales problem opportunities for improvement will be uncovered. The advantages in looks, taste, fat distribution, and soft curd character give the route man a new approach to the customer. It is recommended that homogenized milk be sold as soft curd milk. Educational advertising to groups proved especially effective. Starting with 6% of sales in 1933 distribution increased to 25% of sales in 1937 with homogenized milk selling at a 1¢ premium. E.F.G.

**42. Composition of the Milk from the Breasts of Newly Born Infants.** W. L. DAVIES AND ALLAN MONCRIEFF. National Institute for Research in Dairying, University of Reading, and Middlesex Hospital and Queen Charlotte's Maternity Hospital, London. Biochem. J. *32*, 1238, 1938.

Most infants of both sexes secrete milk when newly born, the secretion being known as witches' or sorcerers' milk. The analyses of five samples of secretions from the breasts of newly-born infants from 8 to 14 days old showed the following values: total solids 7.4–12.5 per cent, consisting mostly of protein and lactose. Compared with normal milk, the secretions are low in lactose but high in globulin and non-protein nitrogen. The secretions contain appreciable amounts of peroxidase and phosphatase. K.G.W.

**43. Ascorbic Acid and Phosphatase Activity.** E. J. KING AND G. E. DELEBY, British Postgraduate School, London. Biochem. J. *32*, 1157, 1938.

It was reported in 1937 (J.B.C. 121, 697, 1937) that ascorbic acid was an intense activator of serum phosphatase. A repetition of these experiments, as well as others, in which significant quantities of ascorbic acid were added to serum, plasma or substrate indicated that ascorbic acid is without effect on the activity of phosphatase. K.G.W.

**44. New Ideas in Milk Drinks.** J. H. FRANDSEN, Mass. State College, Amherst, Mass. Extension Leaflet No. *177*, July, 1938.

Directions are given for making many milk drinks by the addition of fruit juices and other flavoring materials to milk. H.G.L.

**45. The Sales Possibilities of Homogenized Milk.** JAMES R. HUDSON, Baker-Hubbell Dairy, Inc., Peoria, Ill. Ann. Conv. Intern. Assn. Milk Dealers, Sales and Advertising Section, p. 111, 1937.

The advantages of homogenized milk are listed, and a program of public, medical profession, and salesman, education is outlined. A radio and sales promotion contest is described. It is held that the amount of homogenized milk sold will be in proportion to the effort put forth. E.F.G.

## MISCELLANEOUS

**46. How to Control Insects in the Milk Powder Plant.** C. L. FLUKE, Univ. of Wis. Nat. Butter and Cheese Jr. Vol. *29*, No. 15, p. 30, Aug. 10, 1938.

Accumulations of powdered milk in obscure corners or on inaccessible ledges in milk powder plants encourage insect infestation. The most

important insects which use such milk for food are known as carpet beetles (dermestids) such as the black carpet beetle and the Trogoderma beetle. Silver fish (fish moth) may also be found in milk powder factories wherever milk powder is allowed to accumulate. The life histories of these insects are briefly outlined. The prevention and control of these insect infestations is accomplished through general sanitation and cleanliness in the factories. Common places of infection and the most dangerous times of the year are indicated. W.V.P.

**47. Cold Storage Locker Plants.** T. EDWIN THOMAS. Nat. Butter and Cheese Jr. Article I, Vol. *28*, No. 13, p. 10, July 10, 1938. Article II, Vol. *29*, No. 14, p. 6, July 25, 1938.

Article I. The five year development of the cold storage locker idea from an unheard of industry to one with two thousand cold storage locker businesses and investment of nearly $25,000,000 is described. A diagram of a typical cold storage locker plant lay out is shown.

Article II. A cold storage locker plant is described and photographs are presented. Operating expenses for a three hundred and twenty locker plant are itemized. The author estimates a return of 10% on the investment. W.V.P.

**48. The Value of Contests in Stimulating Continued Sales Efforts.** EARLE W. BEEBE, General Sales Manager, Arden Farms, Inc., Los Angeles, Calif. Ann. Conv. Int. Assn. Milk Dealers, Sales and Advertising Section, p. 58, 1937.

Most effective incentives are discussed. Prize money is held to be no incentive but other prizes are effective in causing a man to want to sell. A milk company can afford to spend all the increased gross profit during the effort on prizes. Results of four summer campaigns and the methods of calculating points are given. E.F.G.

**49. Some New Approaches to Accounting for Distribution Costs.** PHILIP SAUNDERS, Pasteur, Charles F. Rittenhouse and Co., Boston, Mass. Annual Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 69, 1937.

The fact that in the milk industry selling and delivery alone accounts for about half of all expense requires that these costs be analyzed and controlled. Many uncontrolled items need to be taken into account in this analysis. Typical milk plant situations and unit costs are given.

E.F.G.

**50. Are Vehicle Records Worth the Cost of Compilation?** F. W. Root, Sales Manager, Glendale Farms, Inc., Wilkes-Barre, Pa. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 63, 1937.

The records are discussed and a classification of items is given.

E.F.G.

**51. A Case Study in Accounting for Capital Assets and Depreciation.** John H. DeVitt, Asst. Auditor, Hammermill Paper Co., Erie, Pa. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 41, 1937.

A punch card system with code numbers has been used effectively in classification and control. This system is described and attention called to its advantages. E.F.G.

**52. A Program of Control of Office Plant Forms.** Charles A. Armitage, Comptroller, United Farmers Cooperative Creamery Association, Boston, Mass. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 32, 1937.

Eliminating needless reports and simplification of others will cut expense without loss of essential information. How forms may be made more usable is discussed. E.F.G.

**53. The Present Status of Social Security Taxes from the Standpoint of Employer Administration.** C. J. Crampton, Executive Secretary, Dallas Chamber of Commerce, Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 8, 1937.

The general application of this legislation to business is considered including accounting methods and responsibilities of the employer.

E.F.G.

**54. Interpreting General Statistics for Dairy Industry Executives.** H. A. Ross, Head of Bureau of Economics, The Borden Co., New York City. Ann. Conv. Intern. Assoc. Milk Dealers, Accounting Section, p. 106, 1937.

Sources and analysis of statistics to answer the following questions are presented

1. What will milk production be?
2. What will be the price for milk?
3. How will general business affect sales?

A comprehensive list of sources of statistical information is given. Suggestions for interpretation are offered. E.F.G.

## PHYSIOLOGY

**55. Studies in the Secretion of Milk Fat. I. The Effect of Inanition on the Blood Lipoids of the Lactating Cow.** J. A. B. SMITH. **II. The Effect of Inanition on the Yield and Composition of Milk Fat.** J. A. B. SMITH AND N. N. DASTUR, Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. Biochem. J. *32*, 1856, 1938.

During the past few years evidence, obtained by simultaneous arterial and venous blood sampling, has shown that the only lipoid fraction of the blood used by the mammary gland for milk fat production is the neutral glyceride fraction and not that comprising the phosphatides or cholesteryl esters. This might suggest that for the secretion of milk fat the gland simply selects from the blood the particular type of glycerides which it requires and that these are then allowed to diffuse into the milk. But it is clear from the composition of the fat itself and from measurements of the activity of the gland that more complex processes, such as synthesis of fat from carbohydrates, are involved. It would appear there are at least two main precursors of milk fat in the blood, the neutral glycerides on the one hand and some form of carbohydrate material on the other. It seems reasonable to suppose the composition of milk fat produced by an individual animal at any particular time will depend on the extent to which each of these precursors contributes toward its production. These two papers are in part a report of experiments to determine the effect of altering blood composition of milk fat.

Inanition of three cows resulted in very slight decreases of considerably less than 10% for the lipoid levels in the corpuscles, during and after a 12-day fast, whereas for the plasma, general reductions of as much as 40% or 50% took place and these low levels persisted even six weeks after free access to food had again been permitted. Neither in the corpuscles nor in the plasma could any appreciable change be detected in the actual nature of the lipoids as a result of inanition, although there was a tendency for greater unsaturation in the plasma fatty acids to accompany milk fever symptoms. It was also shown that if any water soluble fatty acids are present in the non phosphatide fraction of the plasma the amount must be exceedingly small.

Although no appreciable change in the nature of the blood lipoids of the lactating cows during inanition was observed, very considerable changes were observed in the milk fat. The chief change was a decrease of about 80% in the original content of lower acids up to and including $C_{14}$, a deficiency which was almost entirely made good by an increase in the content of oleic acid. A concise review of the theories relating to fat secretion in the light of the above data is included.